

ORIGINAL ARTICLE

New oligochitosan-nanosilica hybrid materials: preparation and application on chili plants for resistance to anthracnose disease and growth enhancement

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New biopolymer oligochitosan (OCS) and OCS/nanosilica (OCS/nSiO₂) hybrid materials with remarkable synergistic effects are likely to be considered natural vaccines for the growth and disease resistance of plants. This study indicated that OCS (50 mg l⁻¹), nSiO₂ (60 mg l⁻¹) and OCS/nSiO₂ (50:50 mg l⁻¹:mg l⁻¹) not only created effective anti-fungal activity in chili plants but also enhanced their growth, even when the plants were infected with *Colletotrichum* sp. After fungal infection, the disease incidence of the control plants increased to 50.3% (after 14 days), 75.7% (after 21 days) and 100% (after 35 days). However, the plants treated with OCS/nSiO₂ were without disease, even when they have been infected by *Colletotrichum* sp. during the 35-day investigation. The fresh weight of shoots and roots, shoot length and chlorophyll content of the plants treated with nSiO₂, OCS or OCS/nSiO₂ hybrid material were higher than those of the control plants. These materials could be considered potential biotic elicitors that not only prevent anthracnose disease but also effectively promote plant growth.

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INTRODUCTION

Chitosan is the second most abundant polysaccharide in nature. It includes monomer glucosamine and acetyl glucosamine units that are linked via a β (1–4) glycosidic bond. The special characteristics of chitosan, such as biodegradability, biocompatibility, nontoxicity, antibacterial, anti-fungal, stimulation of disease resistance and other special characteristics, have led to its wide application in many fields.¹ However, chitosan with high molecular weight obtained from the deacetylation of chitin has limited solubility: it only dissolves in acidic media. Its low solubility in aqueous solvent thus limits its applications.² In recent years, oligochitosan (OCS) obtained from the degradation of chitosan has attracted interest due to its good solubility and some special properties that are superior to those of ordinary chitosan, such as increased disease resistance activity, stimulation of the development of plants or animals, anti-fungal activity, antibacterial activity and high antioxidant activity.^{3–6}

Silicon is the second most abundant element after oxygen in the Earth's crust, and it is found in silicate, alumina silicate and silicon dioxide. Results obtained in 1917 first indicated that silicon has an important role as a necessary nutrient source for plant and animal growth.⁶ It is also considered a physicochemical barrier, as it is

deposited in the walls of the epidermis and vascular tissues of the stem, leaf sheath and hull in most plants. It enhances plant biotic and abiotic resistance,⁷ and plants usually absorb silicon in both mono-silicic and polysilicic acid forms.⁸ Although silicon is abundant in soil, plants are still not sufficiently supported from available silicon sources. In recent years, nanosilica (nSiO₂) from rice husk (an agricultural waste product) has attracted significant research interest, especially as applied to agriculture areas. In 2013, Karunakaran *et al.*⁸ indicated that nSiO₂ synthesized from rice husk was more effective in improving soil characteristics and enhancing the germination of seed than common silica. Manzer *et al.*⁹ also showed that nSiO₂ promoted the germination rate and reduced the germination time in tomato seed and found that its disease resistance was also effectively improved. Thus, nSiO₂ is considered a potential fertilizer for improving harvests.

nSiO₂ and OCS are ecofriendly materials with low costs and special characteristics. Combining OCS with nSiO₂ can produce novel hybrid materials with synergistic characteristics that can be widely applied in many fields. Although there are many studies on chitosan/nSiO₂,^{10–12} OCS/nSiO₂ hybrid material has still not been well researched, other than the study of Yang *et al.*¹³ on the synergistic effect of OCS and silicon on the inhibition of fungal infections by *Monilinia fructicola*,

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which causes brown rot. In this research, we studied the synthesis and characteristics of OCS/nSiO₂ hybrid material applied to agriculture.

EXPERIMENTAL PROCEDURES

Materials

Chitosan from crab shell chitin with a 93.2% degree of deacetylation and Mw of 110 kDa was supplied by the VINAGAMMA center (Ho Chi Minh City, Vietnam). Raw rice husk (RH) was supplied by rice mills in southern Vietnam. H₂O₂ 30% (d: 1.11 g ml⁻¹) was purchased from Merck (Darmstadt, Germany). All other chemicals, including lactic acid, NaOH, HCl, NH₄OH 25% and ethyl alcohol, were of reagent grade. Distilled water was used in all preparations. *Colletotrichum* sp. fungus was provided by the Research and Development Center for Hi-Tech Agriculture Cu Chi (Ho Chi Minh City, Vietnam).

Preparation of OCS

OCS with a molecular weight average of 4.6 kDa was obtained from irradiation of a 4% (w v⁻¹) chitosan/1% (w v⁻¹) H₂O₂ solution, as described by Nguyen *et al.*¹⁴

Heterogeneous degradation of chitosan by oxidation of H₂O₂. Chitosan in flake form was soaked in a glass beaker containing H₂O₂ (1% w v⁻¹) for 24 h. The mixture was poured through a cloth filter, washed several times in distilled water to remove H₂O₂ and dissolved in 1% (w v⁻¹) lactic acid. The solution was neutralized with a 5% (v v⁻¹) NH₄OH solution, and ethyl alcohol was slowly added while stirring. The chitosan precipitate was filtered, washed with alcohol several times and dried at 60 °C in an oven.

Homogeneous degradation of chitosan by radiation γ /H₂O₂. The chitosan obtained after heterogeneous degradation was dissolved by 2% (w v⁻¹) lactic acid, and H₂O₂ and distilled water were added to prepare 4% (w v⁻¹) chitosan/1% (w v⁻¹) H₂O₂ solution. This solution was irradiated in a gamma SVST Co-60/B irradiator (Isotopes Institute, Budapest, Hungary) with an absorbed dose of 21 kGy to prepare OCS with an average molecular weight of 4.6 kDa. After irradiation, the solution was neutralized with NH₄OH (5% aqueous). OCS was precipitated in absolute alcohol, washed with alcohol several times and dried in an oven at 60 °C.

Preparation of nSiO₂ from rice husk

The process of nSiO₂ preparation was adopted from a method described in previous reports^{15–17} with some modifications. RH was rinsed with water to remove dust, soluble substances and other contaminants. It was dried at 60 °C in a forced air oven, and 50 g of dried RH was treated with 500 ml of 1% HCl at room temperature for 2 h by magnetic stirring. It was kept intact overnight, decanted and thoroughly washed with distilled water. The product was ground into powder, and the obtained RH was incinerated at 700 °C for 2 h inside a programmable furnace to obtain nSiO₂.

Preparation of OCS/nSiO₂

A glass beaker containing 1 g of nSiO₂ was added to 7.7 ml of 1 M NaOH to produce Na₂Si₃O₇ (Na₂SiO₃·2SiO₂) and stirred for 30 min. Then, 25 ml of 4% (w v⁻¹) OCS and distilled water was slowly added to reach a final volume of 100 ml of OCS/nSiO₂. The mixture was stirred for 4 h at room temperature. The pH of the mixture was adjusted to 5.0, 6.5 or 8.5 by the addition of 1 M HCl or 1 M NaOH. The OCS/nSiO₂ solutions were poured into glass bottles and kept intact at room temperature for 24 h to investigate the stability of the prepared hybrid mixtures of OCS/nSiO₂.

Characterization of OCS

The Mw of OCS was measured using an Agilent GPC (LC-20AB; Shimadzu, Kyoto, Japan) with an RI-10A detector and 250 ultra-hydrogel column from Waters (Milford, MA, USA). The calibration standard for the columns was pullulan with a Mw of 780–380 000 Da. The eluent was an aqueous solution of 0.25 M CH₃COOH/0.25 M CH₃COONa with a flow rate of 1 ml min⁻¹.¹⁸ The chemical structure of OCS, nSiO₂ and hybrid material was analyzed using an FT-IR 8400S spectrometer (Shimadzu) and KBr pellets.

Characterization of nSiO₂

Silica content and the amount of metallic impurities in the sample were analyzed using an energy dispersive X-ray spectrometer, Horiba 7593-H (Horiba, Kyoto, Japan). The X-ray diffraction (XRD) pattern of nSiO₂ was recorded on an X-ray diffractometer, D8 Advance A25 (Bruker, Karlsruhe, Germany). The particle size and morphology of the nSiO₂ and hybrid were investigated using transmission electron microscopy, model JEM1400 (JEOL, Tokyo, Japan).

Effect of growth enhancement and anthracnose disease resistance of OCS, nSiO₂ and OCS/nSiO₂ hybrid material on chili plants

The 60-day-old chili plants (*Capsicum frutescens* L.) were divided into four lots:

Lot 1 and lot 2 were control plants. In lot 1, chili plants were treated with water and not infected by *Colletotrichum* sp. fungus. In lot 2, the chili plants were treated with water and infected with *Colletotrichum* sp. fungus.

The chili plants in lot 3 and lot 4 were treated with OCS (nSiO₂ or hybrid). In lot 3, plants were sprayed with OCS (nSiO₂ or hybrid) and not infected with *Colletotrichum* sp. In lot 4, plants were sprayed with OCS (nSiO₂ or hybrid) before being infected with *Colletotrichum* sp.

Treatment of OCS (nSiO₂ or hybrid) involved spraying (1 week/time) in the afternoon, when it was cooler. The first artificial infection of chili plants with *Colletotrichum* sp. occurred one day after the second OCS (nanosilica or hybrid) spraying. The second infection occurred a week later. The chili plants continued to grow for three additional weeks, and the fresh weight, dried weight, chlorophyll content and the disease incidence (%) were investigated.

Statistical analysis

The data obtained from three replicates were subjected to analysis of variance. Differences among treatments were analyzed, with $P < 0.05$ considered significant according to Duncan's multiple range tests. Statistical procedures were performed using the software package SAS 9.1.3 for Windows (SAS Institute, Toronto, ON, Canada).

RESULTS AND DISCUSSION

Characteristics of nSiO₂

Energy dispersive X-ray spectrometer. Based on previous reports,^{15–17} we treated rice husk (RH) with HCl before incinerating to remove inorganic impurities such as Fe, Mn, Ca, Na, K and Mg that can affect the material properties (Figure 1). Alkali metals are the main cause of melting surfaces and promote the crystallization of amorphous silica.^{15–19}

According to studies of the synthesis of nSiO₂ from Vietnam rice husk using sol-gel method of Le *et al.*,²⁰ Vietnam rice husk ash that is not treated with an acid solution is K₂O (0.39%), Al₂O₃ (0.48%), Fe₂O₃ (0.15%), CaO (0.73%), MgO (0.55%), Na₂O (0.12%) and SiO₂ (96.15%). In this research, the original material was Vietnam rice husk, not rice husk ash, but the content was the same. The HCl concentration used to treat RH was 1% (w w⁻¹), lower than that of HCl used by Wang *et al.*,¹⁵ and the experiment was performed at room temperature. Collected silica samples from RH1 powder were analyzed by energy dispersive X-ray spectrometer to estimate the silica content and metallic impurities in the sample, and the result shows that RH1 contained only 44.77 wt% Si and 55.23 wt% O. Thus, HCl 1% (w w⁻¹) treatment of RH in 2 h at room temperature effectively removed the metallic impurities. Additionally, the combustion of RH after HCl treatment at 700 °C in 2 h removed all organic components in RH (cellulose, hemicellulose and lignin), and the resulting nSiO₂ sample formed a white powder.

FT-IR analysis. RH consists of 70–85% organic components and the rest consists of inorganic materials, depending on variable species, climate and geography.²¹ Because the main components in RH are organic, the Fourier-transform infrared spectroscopy (FT-IR) results

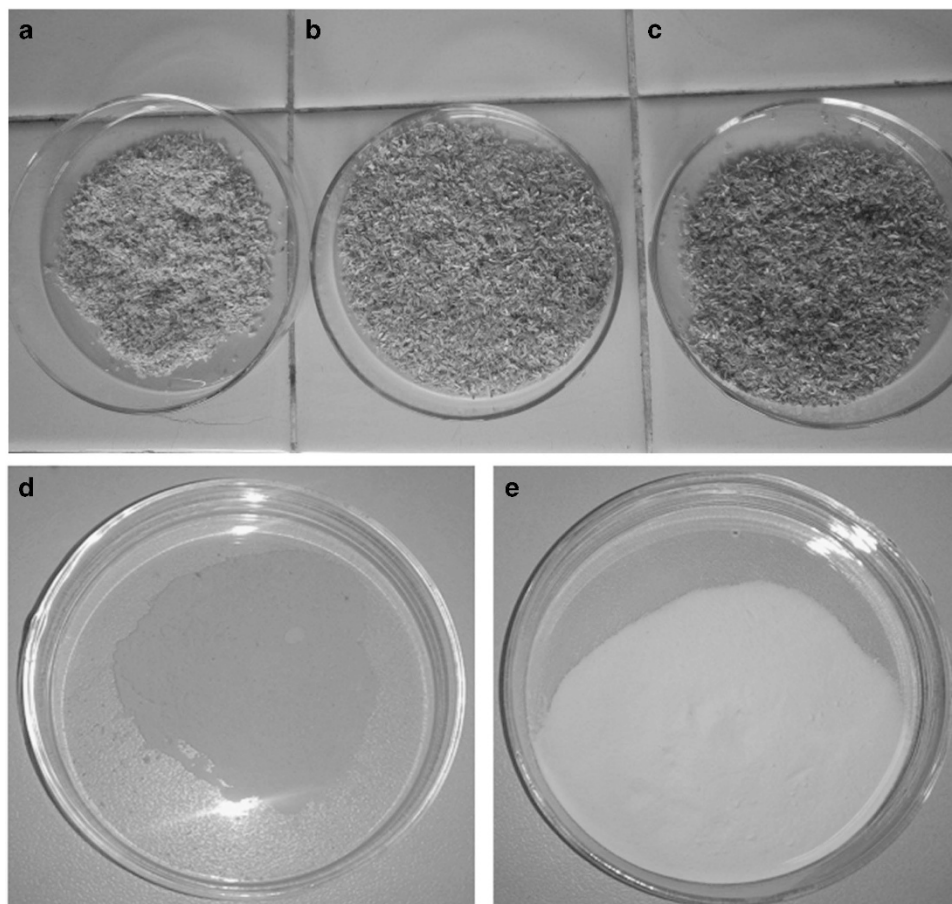


Figure 1 Rice husk: (a) origin (raw rice husk (RH)), (b) washed with distilled water, (c) treated with HCl 1% (RH1), (d) treated by HCl 1% and ground and (e) nanosilica (nSiO₂). A full color version of this figure is available at the *Polymer Journal* journal online.

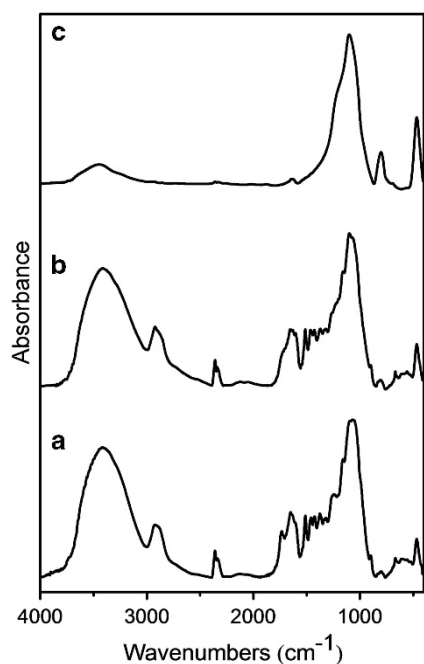


Figure 2 Fourier-transform infrared spectroscopy (FT-IR) spectrum of original rice husk (raw rice husk (RH)) (a), RH after treating with HCl 1% (RH1) (b) and nanosilica (nSiO₂) (c).

for RH had characteristic peaks for cellulose, hemicellulose and lignin (Figure 2). At 3400 cm⁻¹, there was a broad, high peak assigned to O-H stretching in absorbed water or OH groups in cellulose, lignin and hemicellulose. The peak at 2921 cm⁻¹ was a stretching vibration for C-H. There was also a peak at 1733 cm⁻¹ (C=O in free carbonyl groups, acetyl or carboxylic acid in hemicellulose). The peaks at 1650, 1600 and 1512 cm⁻¹ were vibrations of C=C in the aromatic ring (lignin), while those at 1460, 1425 and 1375 cm⁻¹ characterized vibrations of CH₂ and CH in lignin, cellulose and hemicellulose. The peaks at 1320 and 1240 cm⁻¹ were vibrations of O-H in phenol (cellulose) and the guaiacyl ring breathing with CO stretching (lignin and hemicelluloses) of esters, respectively. A low peak at 1160 cm⁻¹ and a high peak at 1070 cm⁻¹ were assigned to C-O-C (carbohydrate) and C-O in primary alcohol, guaiacyl (lignin), respectively.²²⁻²⁴

Although RH was treated with acid, FT-IR spectrum of RH1 sample still had characteristic peaks for organic components. However, peaks at 1733 and 1240 cm⁻¹ nearly disappeared, indicating that ester and carboxyl in RH can be degraded by acid treatment.

Comparing to the IR spectrum of RH and RH1, the IR spectrum of SiO₂ obtained after incinerating RH1 at 700 °C for 2 h clearly shows that the peaks of organic materials in RH completely disappeared. At 3440 cm⁻¹, a broad, high peak was related to hydroxyl group stretching, and the small, low peak that appeared at 1637 cm⁻¹ was assigned to the bending vibration of water molecules absorbed on silica particle surfaces, in agreement with the results of Martinez *et al.*²⁵ Bands near 1101, 802 and 438 cm⁻¹ were assigned to

the bending and stretching vibration of O-Si-O.¹⁵ The peaks at 1101 and 1200–1250 cm^{-1} were attributed to the asymmetric stretching mode of SiO_4 coordination units.^{25,26}

X-ray diffraction. The degree of crystalline form of silica was analyzed by XRD, and the XRD pattern of silica sample is shown in Figure 3, with only one broad peak at $2\theta = 21\text{--}22^\circ$. This confirmed the purity and amorphous structure of nSiO_2 generated from combusting acid-treated RH powder at 700 $^\circ\text{C}$.

Silica can crystallize in different phases depending on temperature and pressure. The order of changing phase of silica is as follows: silica is α -quartz, β -quartz (573 $^\circ\text{C}$), β -tridymite (870 $^\circ\text{C}$) and SiO_2 forms β -cristobalite at 1470 $^\circ\text{C}$. At ~ 700 $^\circ\text{C}$, amorphous silica was generated, whereas the crystalline phases usually formed above this temperature.²⁷ Thus, the XRD results affirmed that 700 $^\circ\text{C}$ was a suitable temperature for generating amorphous SiO_2 .

Transmission electron microscopy. The particle shape and size of nSiO_2 obtained from rice husk were analyzed using transmission electron microscopy; the transmission electron microscopy graph and diagram of particle size distribution in Figure 4 shows spherical silica particles, which tended to form clusters. The average size of silica particles was $\sim 30\text{--}50$ nm.

Synthesis of OCS/nSiO₂ hybrid material

Effect of pH on synthesis of OCS/nSiO₂ hybrid material. Based on studies of the chitosan/ nSiO_2 material,^{28–33} we prepared to synthesize OCS/ nSiO_2 hybrid material from OCS solution and nSiO_2 with a weight ratio of 1:1 (w w^{-1}), and the solution pH was adjusted from 5.0 to 8.5. OCS/ nSiO_2 was a homogenous gel mixture, but during aging at room temperature over 24 h, the samples prepared at pH 5.0 and 6.5 clearly precipitated. Precipitation was also observed for the OCS/ nSiO_2 sample with pH 8.5, but agglomeration was not as clear; longer times were needed to observe precipitation. The OCS/ nSiO_2 sample with pH 7.5 not only did not precipitate over 24 h at room temperature, or even for longer time, but agglomeration did not occur. Thus, pH 7.5 is suitable for obtaining a stable OCS/ nSiO_2 gel.

Witoon *et al.*³² studied the effect of chitosan concentrations and pH on silica precipitation and morphology; the results indicated that with a pH range of 3.0–6.0 and chitosan/silica with a mass ratio of 0.8–1.6 heated at 40 $^\circ\text{C}$, chitosan and silica generated a homogenous gel. However, according to Tiraferri *et al.*,³⁴ the mechanism of absorption of chitosan on silica showed that chitosan and silica effectively

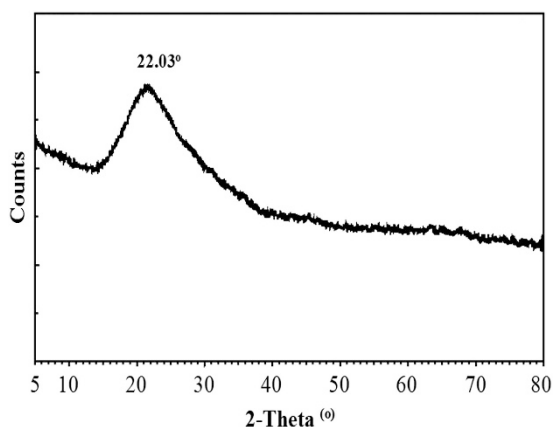


Figure 3 X-ray diffraction (XRD) pattern of nanosilica (nSiO_2) obtained from incinerating raw rice husk (RH) treated with HCl 1%.

interacted and that the absorbed mass of chitosan on surface of silica was greatest in medium at pH from 7.0 to 8.0, whereas the absorption of chitosan on silica decreased at $\text{pH} > 8.0$. This is in agreement with our study on the effect of pH on the stability of OCS/ nSiO_2 .

In this study, the initial pH of the OCS solution was 4.0, and in acid medium, positively charged amino groups were present on the backbone chains of the OCS molecules. The nSiO_2 surface was negative because it was alkalinized before mixing with the OCS solution. After mixing, the pH of OCS increased, whereas that of silica decreased. At pH 5.0 and 6.5, amino groups on OCS were still protonated, but the charge density of silica surface decreased. Thus, the interaction between OCS and silica through ionic crosslinking interactions declined, and the absorbed OCS on silica was low. This may result in the precipitation of OCS/ nSiO_2 at pH 5.0 and 6.5. With increasing pH, the free ammonium groups of OCS were progressively neutralized, and the repulsion among the absorbed OCS molecule decreased. This led to accumulated polyelectrolytes on the surface. At the same time, the silanol dissociation of silica increased, and the surface charge density thus increased. This provided electrostatic interactions between OCS and nSiO_2 . OCS was also absorbed irreversibly at the silica interface through hydrogen interactions, and OCS and nSiO_2 existed stably at pH 7.5. According to Tiraferri *et al.*,³⁴ the chitosan charge was zero at pH 8.0, but above pH 8.0, the polyelectrolyte of chitosan backbone was negative. This may have led to electrostatic repulsion between chitosan and the silica surface and could explain the appearance of separation in samples at pH 8.5.

Transmission electron microscopy. To investigate the morphology of the OCS/ nSiO_2 hybrid material, samples at pH 5 and 7.5 were analyzed by TEM methods; the results are shown in Figures 5 and 6. They indicated that nSiO_2 particles distributed in OCS solution at medium conditions at pH 7.5 were small. The particle size was $\sim 8\text{--}10$ nm, and the silica particle shape differed from the original particles. This may have been due to linking between very small nSiO_2 particles. In contrast, nSiO_2 particles in a mixture at pH 5.0 were clearly separated, and the particles were larger than those in the mixture at pH 7.5. In this case, the particle size distribution of nSiO_2 was $\sim 10\text{--}15$ nm. This result indicated that the OCS/ nSiO_2 bulk gel was not stable at pH 5.0, the interaction between OCS and nSiO_2 was not good, and the nSiO_2 particles tended to produce larger clusters.

Fourier-transform infrared spectroscopy. The FT-IR spectra of OCS shows characteristic absorptions: peaks at 3200–3500 cm^{-1} (O-H, N-H), 1647 cm^{-1} (amid I), 1593 cm^{-1} (amid II), 1417 cm^{-1} (symmetrical deformation of $-\text{CH}_3$ and $-\text{CH}_2$), 1319 cm^{-1} assigned to the absorbance of C-N of $\text{CH}_3\text{CONH-}$ (amid III) and the peaks at 1074, 1031, 1153–895 cm^{-1} assigned to the stretching vibration of C-O, the stretching vibration of C-O-C in the glucose ring and the special peaks for the $\beta(1\text{--}4)$ glycoside bond in chitosan, respectively.

Compared to the FT-IR spectra of OCS and nSiO_2 , the FT-IR of OCS/ nSiO_2 in Figure 7 shows characteristic absorptions of both OCS and nSiO_2 . However, there was a shift in characteristic peaks, which confirms the successful preparation of hybrid material. The interaction of OCS with nSiO_2 narrowed the broad band of the O-H, N-H stretching vibrations of OCS at 3400 cm^{-1} and its intensity was higher than that for the initial OCS. In addition, bands at 1083 and 781 cm^{-1} assigned to the vibration of Si-O-C appeared, which were not visible in pure OCS and nSiO_2 . A new peak at 927 cm^{-1} , related to the Si-OH bonds, was observed because of the formation of H bonds between the silanol groups of the silica network and the amide and oxy groups of OCS³⁵ (Figure 8).

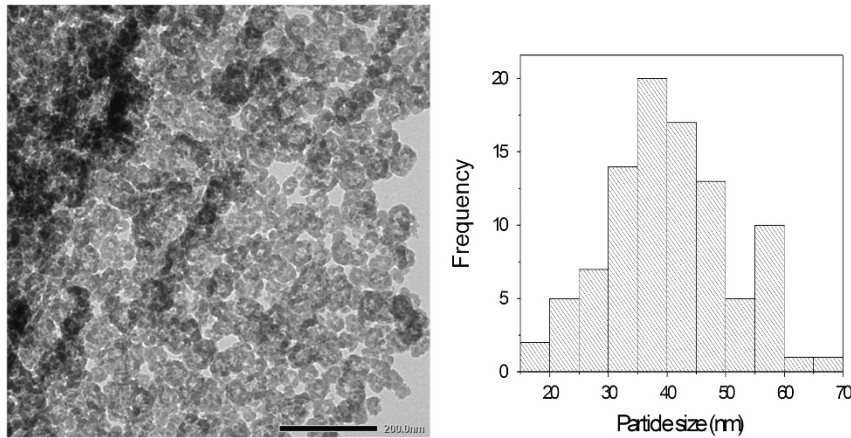


Figure 4 Transmission electron microscopy (TEM) micrograph and diagram of particle size distribution of nanosilica ($nSiO_2$) obtained from incinerating raw rice husk (RH) treated by HCl 1%.

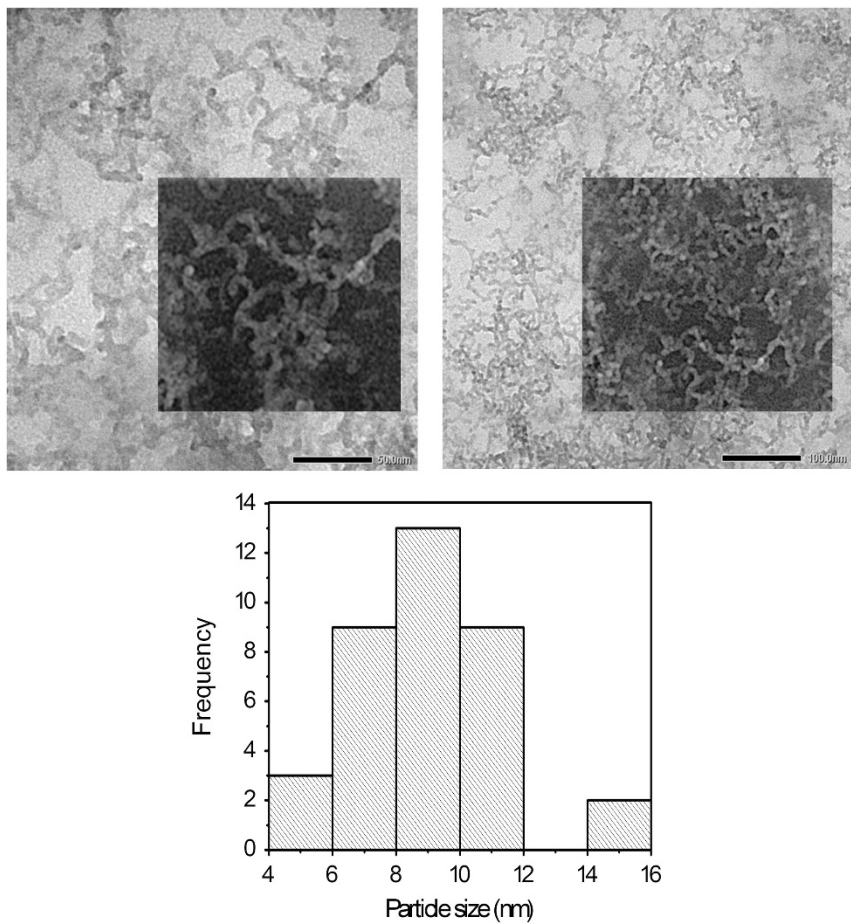


Figure 5 Transmission electron microscopy (TEM) micrograph and diagram of particle size distribution of oligochitosan/nanosilica (OCS/ $nSiO_2$) (pH = 7.5).

Investigation into effects of OCS, $nSiO_2$ and OCS/ $nSiO_2$ on the growth enhancement of chili plants

Effect of the growth enhancement of OCS on chili plants. The results in Table 1 indicate that chili plants treated with OCS solutions with different concentrations of 25–100 $mg\ l^{-1}$ had higher shoot and root weight than did control plants. The OCS solution at 50 $mg\ l^{-1}$ most effectively promoted the growth of plants. The fresh weight of watered plant shoots was 25.67 g per plant, whereas the plant treated with OCS

at 50 $mg\ l^{-1}$ had a fresh weight of 30.45 g per plant. The OCS also stimulated root growth. The fresh weight of root was 5.23 g per root when plants were sprayed with 50 $mg\ l^{-1}$ OCS solution. Without OCS, the fresh weight of roots was only 3.52 g per root. Thus, OCS effectively promotes chili plant biomass.

In addition to the biomass factor, the length and chlorophyll content of chili plants increased in the presence of OCS (Table 1). Plants were treated with OCS at a concentration of 25, 50, 75 and

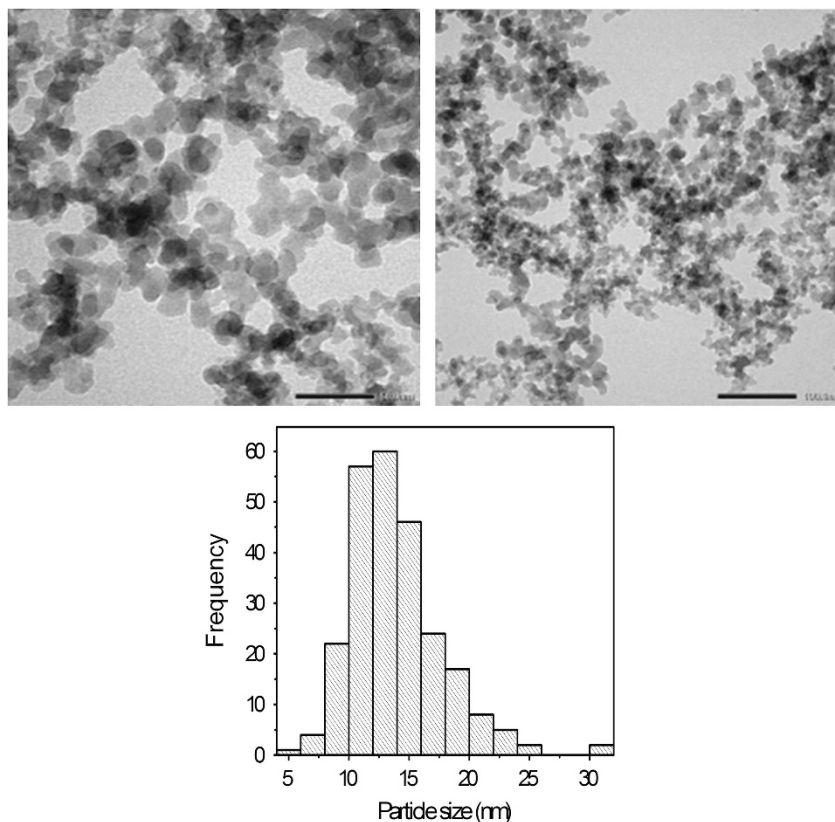


Figure 6 Transmission electron microscopy (TEM) micrograph and diagram of particle size distribution of oligochitosan/nanosilica (OCS/nSiO₂) (pH = 5.0).

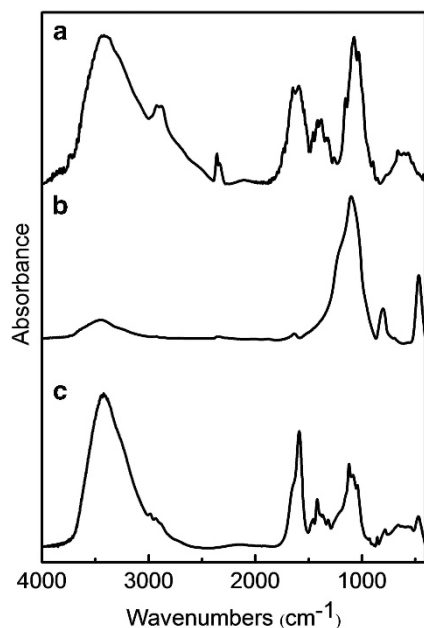


Figure 7 Fourier-transform infrared spectroscopy (FT-IR) spectrum of oligochitosan (OCS) (a), nanosilica (nSiO₂) (b) and OCS/nSiO₂ hybrid material (c).

100 mg l⁻¹, and the measured average plant length was 62.25, 68.75, 64.00 and 65.00 cm per plant, respectively. OCS stimulated a 1.2-fold increase in plant length compared with the control sample. The chlorophyll content showed a similar trend as plant length. The plants

without OCS had a chlorophyll content of 21.29 mg g⁻¹, whereas the chlorophyll content of the plants treated with OCS of 25, 50, 75 and 100 mg l⁻¹ was 36.95, 36.30, 32.28 and 28.95 mg g⁻¹, respectively. These results also indicated that OCS affected the growth enhancement of chili plants and that the highest effect of stimulation in growth was observed at an OCS concentration of 50 mg l⁻¹.

Effect of the growth enhancement of nSiO₂ on chili plants. nSiO₂ (30–50 nm) obtained from rice husk was dispersed in water to produce solutions of different concentrations that were sprayed on chili plants. Table 2 shows that compared with the plant control, nSiO₂ with a concentration of 40–100 mg l⁻¹ stimulated an increase in chili plant biomass and nSiO₂, as a concentration of 60 mg l⁻¹ produced the best growth effects for both plant shoots and roots. The plants treated with water (control) had a fresh weight of 25.67 g per plant (in the case of stem) and 3.52 g per root (in the case of root). The fresh weight of the shoots of plants treated with nSiO₂ at 40, 60 and 100 mg l⁻¹ increased by 12.12%, 17.53% and 9.70%, respectively. The fresh weight of roots also increased by 15.54%, 89.85% and 77.08% compared with the weight of plants without nSiO₂.

In addition, a significant increase in the length and chlorophyll content of plants in the presence of nSiO₂ was observed, and the result is shown in Table 2. The length of chili plants treated with nSiO₂ of 40, 60 and 100 mg l⁻¹ increased by 10.71%, 13.70% and 3.98% compared to plants treated with water. In the control sample, the chlorophyll content was 21.29 mg g⁻¹, whereas the plant sprayed with nSiO₂ (60 mg l⁻¹) had a chlorophyll content of 30.67 mg g⁻¹. These results indicated that nSiO₂ significantly affected the growth enhancement of chili plants and that the nSiO₂ solution at a concentration of 60 mg l⁻¹ had the most effective growth stimulation in the chili plant.

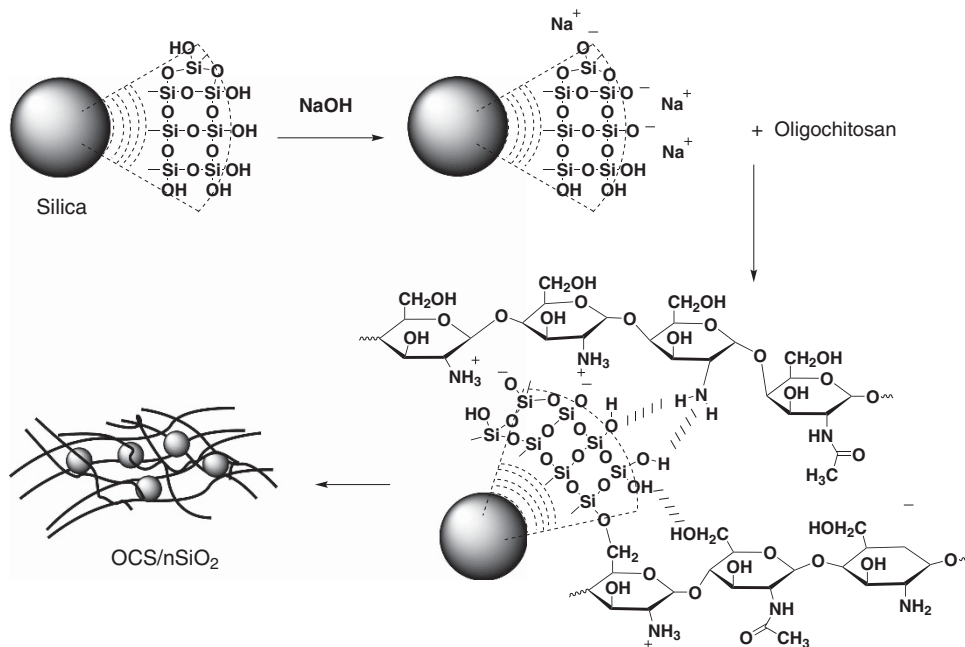


Figure 8 Illustration of interactions between oligochitosan (OCS) and nanosilica (nSiO₂).

Table 1 Fresh weight, shoot length and chlorophyll content of chili plants treated with OCS

OCS conc. (mg l ⁻¹)	Fresh weight (g per plant)		Shoot length (cm per plant)	Total chlorophyll (a+b) content (mg g ⁻¹)
	Shoot biomass	Root biomass		
0 (water)	25.67 ± 0.83	3.52 ± 0.55	56.00 ± 0.98	21.29 ± 0.51
25	26.56 ± 0.48	4.23 ± 0.93	62.25 ± 2.33	36.95 ± 3.67
50	30.45 ± 1.41	5.23 ± 1.13	68.75 ± 6.56	36.30 ± 1.04
75	26.78 ± 2.32	5.12 ± 0.87	64.00 ± 5.06	32.28 ± 2.64
100	27.89 ± 1.21	4.56 ± 1.08	65.00 ± 1.66	28.95 ± 3.47
LSD _{0.05}	0.65	0.19	3.32	1.04
CV (%)	1.26	2.22	2.79	1.77

Abbreviations: CV, coefficient of variation; LSD, least significant difference; OCS, oligochitosan.

Table 2 Fresh weight, shoot length and chlorophyll content of chili plants treated with nSiO₂

nSiO ₂ conc. (mg l ⁻¹)	Fresh weight (g per plant)		Shoot length (cm per plant)	Total chlorophyll (a+b) content (mg g ⁻¹)
	Shoot biomass	Root biomass		
0 (water)	25.67 ± 0.83	3.52 ± 0.55	56.00 ± 0.98	21.29 ± 0.51
40	28.78 ± 3.09	4.24 ± 1.54	62.00 ± 0.88	24.61 ± 0.61
60	30.17 ± 3.34	4.59 ± 0.60	63.67 ± 1.54	30.67 ± 1.56
100	28.16 ± 2.09	4.14 ± 0.58	58.23 ± 4.62	25.42 ± 1.17
LSD _{0.05}	0.13	0.61	2.44	0.55
CV (%)	0.23	7.42	2.15	1.01

Abbreviations: CV, coefficient of variation; LSD, least significant difference; nSiO₂, nanosilica.

Effect of growth enhancement of OCS/nSiO₂ hybrid material on chili plants. According to the above results, 50 mg l⁻¹ was the most suitable concentration of OCS, with the most positive impacts on the growth enhancement of chili plant, whereas the most effective concentration of nSiO₂ on growth enhancement was 60 mg l⁻¹. Based on previous studies on chitosan/nSiO₂,^{28–30} the OCS/nSiO₂ hybrid material in this study was created from an OCS solution and nSiO₂ with a weight ratio of 1:1 (w w⁻¹). This ratio was suitable for generating a stable gel. Thus, an OCS/nSiO₂ mixture (50 mg l⁻¹:50 mg l⁻¹) was used in this investigation.

Compared with control plants, the fresh weights of stem and root of plants sprayed with OCS/nSiO₂ hybrid material were higher, at 30.23 g plant⁻¹ (biomass of stem) and 4.78 g per root (biomass of root), whereas the fresh weights of stem and root of the control plants were 25.67 g per plant and 3.52 g per root (Table 3). The length and chlorophyll content in Table 3 also indicated that using OCS/nSiO₂ had a great influence on the growth enhancement of chili plants. Comparing the results from hybrid material to those from separate

components showed that the combination of OCS and nSiO₂ created a mixture with synergistic activity that can effectively enhance the growth of chili plants.

Effect of OCS/nSiO₂ on resistance to chili anthracnose disease and the growth of chili plants caused by *Colletotrichum* sp.

OCS/nSiO₂ (10 g l⁻¹:10 g l⁻¹) was prepared as in the above description. The mixture was diluted with water to produce a solution of 50 mg l⁻¹ OCS:50 mg l⁻¹ nSiO₂, and this solution was used to treat chili plants as an elicitor. The OCS/nSiO₂ hybrid material resistance activity to anthracnose disease caused by *Colletotrichum* sp. in Table 4 showed that OCS/nSiO₂ had effective anti-fungal activity on chili plant. In particular, after infecting the germ, the disease incidence of the control plants increased to 50.3% (after 14 days), 75.7% (after 21 days) and reached 100% after 35 days. However, for the plants treated with the OCS/nSiO₂ mixture, regardless of whether the plants were infected, they were not completely diseased with *Colletotrichum* sp. during the 35-day investigation.

Table 3 Fresh weight, shoot length and chlorophyll content of chili plants treated with OCS/nSiO₂

Sample	Fresh weight (g per plant)		Shoot length (cm per plant)	Total chlorophyll (a+b) content (mg g ⁻¹)
	Shoot biomass	Root biomass		
Control (water)	25.67 ± 0.83	3.52 ± 0.55	56.00 ± 0.98	21.29 ± 0.51
OCS/nSiO ₂ (50 mg l ⁻¹ : 50 mg l ⁻¹)	30.23 ± 2.33	4.78 ± 0.82	71.33 ± 2.11	32.79 ± 1.06
LSD _{0.05}	1.50	0.33	1.49	0.63
CV (%)	1.21	4.13	2.88	1.22

Abbreviations: CV, coefficient of variation; LSD, least significant difference; nSiO₂, nanosilica; OCS, oligochitosan.

Table 4 Disease incidence (%) of plants treated with OCS/nSiO₂

Sample	Disease incidence (%)		
	14 days	21 days	35 days
Control (sprayed water), not infected	0	0	0
Control (sprayed water), infected	50.3 ± 2.6	75.7 ± 3.8	100
OCS/nSiO ₂ (50 mg l ⁻¹ : 50 mg l ⁻¹), infected	0	0	0

Abbreviations: nSiO₂, nanosilica; OCS, oligochitosan.

Anthrax disease caused by *Colletotrichum* sp. is a disease that causes serious damage to the economies of many countries. It affects shoots, causes rotten plant tops and damages different parts of the plant and fruits; if the disease spreads, the plant will gradually die. Infection of the plant with *Colletotrichum* sp. from the experiments showed that anthrax disease rather seriously affected the growth of control plants. In this result, the fresh weight of plants decreased from 25.56 g per plant (the sample was not infected) to 17.56 g per plant after a 35-day infection. Disease treatment with OCS/nSiO₂ (50 mg l⁻¹:50 mg l⁻¹) not only improved the growth of uninfected plants but also promoted the development of infected plants. The fresh weight and length of the plants uninfected and plants infected with *Colletotrichum* sp. after being treated with an OCS/nSiO₂ mixture were nearly similar: the fresh weight was ~30 g per plant, and the length was ~71 cm per plant. The fresh weight and length of the infected plants sprayed with OCS/nSiO₂ hybrid material were higher than those of infected control plants, at 17.56 g per plant (fresh weight) and 47.33 cm per plant (length). The chlorophyll of the infected plants sprayed with OCS/nSiO₂ was 34.05 mg g⁻¹ more than 20 mg g⁻¹ for the infected control plants, indicating that the OCS/nSiO₂ mixture (50 mg l⁻¹:50 mg l⁻¹) produced a high growth enhancement of chili plants, even when they were infected with *Colletotrichum* sp. Thus, the OCS/nSiO₂ hybrid material could be considered a potential biotic elicitor that not only prevents anthrax disease but also effectively promotes the growth of plants.

Although the mechanism of OCS/nSiO₂ hybrids on the growth enhancement of plants and a synergistic effect of anti-fungal activity has not been specifically researched, the mechanism of each component on preventing plant diseases was suggested. According to previous research,³⁶ OCS is effective in several plant-disease interactions via the following processes: binding proteins to OCS on plants at signal perception processes, allowing entry into the plant nucleus and the ability to act directly at the chromatin level. The OCS signal enters the cytoplasm, and the OCS signaling transmits quickly and develops a complicated signaling network at the signal-transduction process. This indicates that OCS can elicit plant hormones, especially jasmonic acid production, in plants while salicylic acid and jasmonic acid are

essential plant hormones required for signal transduction leading to plant resistance. In addition, defense-related proteins, enzymes and activity such as glucanase and chitinase can be induced. In defense-related secondary metabolite accumulation, OCS can create pisatin (a phytoalexin) with anti-fungal activities and elicit lignin production. The plant cell wall will strengthen via the deposition of lignin; simultaneously, the synthesis of lignin precursors such as ferulic, *p*-coumaric and sinapic acids, which have anti-microbial activity similar to benzoic, caffeic, protocatechuic and chlorogenic acids, was stimulated by OCS treatment. In addition to OCS's activities, nSiO₂ enhances the resistance of plants to diseases via two mechanisms: Si acts as a physical barrier by depositing beneath the cuticle to form a cuticle-Si double layer. This layer can mechanically prevent the fungal penetration of plants, thereby avoiding infection. Another recently proposed mechanism is that soluble Si acts as a modulator of host resistance to pathogens by producing phenolics and phytoalexins in response to fungal infection and enhancing the activity of chitinases, peroxidases and polyphenoloxidases that limit colonization by the fungus.^{6,7}

CONCLUSIONS

This study describes the synthesis of hybrid material from low-cost, abundant waste products such as OCS from the chitosan and chitin components in crab shell or cuttlebone and nSiO₂ from rice husk; it is likely to create a novel material that possesses special synergistic characteristics of both components. Plants treated with OCS/nSiO₂ were not completely diseased with *Colletotrichum* sp. The shoot biomass, root biomass, length and chlorophyll content of plants with treated OCS, nSiO₂ or OCS/nSiO₂ were higher than those of the control plants.

These new materials are not only valuable and useful for many application areas, especially agriculture, but they can also help solve environmental pollution problems. They are environmentally friendly agrochemical products that can be substituted for the toxic chemical components that are currently used in agriculture.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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