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Novel thiosemicarbazone chitosan derivatives: Preparation, characterization, and antifungal activity

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ABSTRACT

Three novel thiosemicarbazone chitosan derivatives were obtained via condensation reaction of thiosemicarbazide chitosan with phenylaldehyde, o-hydroxyphenylaldehyde, and p-methoxyphenylaldehyde, respectively. Antifungal activity against the common crop-threatening pathogenic fungi *Stemphylium solani weber* (*S. solani*), *Rhizoctonia solani Kühn* (*R. solani*), *Alternaria solani* (*A. solani*), and *Phomopsis asparagi* (*Sacc.*) (*P. asparagi*) was tested in vitro at 0.05, 0.1, and 0.5 mg/mL. The derivatives had broad-spectrum antifungal activity that was greatly enhanced in comparison with chitosan. In fact, the highest antifungal index reached 100%. At 0.05 mg/mL, the o-hydroxyphenylaldehyde thiosemicarbazone chitosan inhibited growth of *R. solani* at 52.6%, and was stronger than polyoxin whose antifungal index was found to be 31.5%. The chitosan derivatives described here lend themselves to future applicative studies in agriculture.

1. Introduction

Plant diseases can cause death of crops with great economic losses. Particularly, those diseases are usually caused by pathogenic fungi. For example, *Stemphylium solani weber*, a common crop-threatening fungus, has become a threat to tomato production in some regions (Cedeño & Carrero, 1997). *Rhizoctonia solani Kühn*, is a pathogen which brings great dmages to crops (Elad, Hadar, Chet, & Henis, 1982). Early blight, caused by the fungus *Alternaria solani*, is one of the most serious foliar diseases of tomato (Maiero, Bean, & Ng, 1991). Stem blight caused by *Phomopsis asparagi* (*Sacc.*) has been a great threat to asparagus (Uecker, 1991). Chemical fungicide was considered to be one of the most effective tools that control pathogenic diseases. However, the frequent and wide application of traditional fungicides has caused serious problems such as environmental pollution and threats to human health. Therefore, it is critically necessary to discover new fungicidal alternatives.

Chitosan was the deacetylated derivative of chitin which obtained from the shells of Crustacean and cell walls of some fungi such as *Aspergillus niger* (Muzzarelli, 1983; Muzzarelli et al., 2011). It was proved to have broad-spectrum antifungal activity against a variety of fungi (Bautista-Baños et al., 2006; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). Early in 1979, Allan and Hadwiger (1979) found chitosan had better fungicidal activity than chitin. Of the 46 tested fungi, chitosan showed inhibitory effect on 32 isolates. Bautista-Baños, Hernández-López, Bosquez-Molina, and Wilson (2003) evaluated the in vitro fungicidal effect of chitosan on the development of *Colletotrichum* (*C.*) *gloeosporioides.* At concentrations of 2.5% and 3%, chitosan can completely inhibit growth of the fungus. El Ghaouth, Arul, Asselin, & Benhamou (1992) found that the inhibitory effect on *Alternaria alternata*, *Botrytis cinerea*, *C. gloeosporioides*, and *Rhizopus stolonifer* enhanced with increasing concentration (0.75–6.0 mg/mL) of chitosan. However, the use of chitosan was limited due to its poor solubility and weaker antifungal activity compared with commercial fungicide. Hence, lots of researchers tried to improve both solubility and antifungal activity of chitosan via chemical modification. New derivatives such as chitosan quaternary ammonium salt, thiourea, N-alkyl and so on were prepared. For example, Muzzarelli et al. (1990) reported NCB-chitosan synthesized to overcome the solubility problems and used with 298 microbial strains. Guo et al. (2007a) reported antifungal activity of a series of quaternized chitosan derivatives. Compared with chitosan, all the quaternized chitosan derivatives have better antifungal activities, and the max inhibitory index was 86.7% in vitro. Eweis, Elkholy, and Elsabee (2006) prepared a chitosan thiourea derivative (TUCS). Its antifungal activity against *R. solani*, *Sclerotium rolfsii*, and *Fusarium solani* was investigated in vitro. The prepared thiourea derivative had a significant inhibitory effect on the investigated fungi at the concentrations of 5–1000 μg/mL. Rabea et al. (2006) reported the

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preparation of N-alkyl chitosan (NAC) derivatives. Their fungicidal activity against *B. cinerea* and *Pyricularia grisea* was evaluated by a radial growth bioassay. Most of the NAC derivatives showed better activity against the fungi than chitosan itself. The most active derivative was N-(2,2-diphenylethyl)chitosan, of which EC_{50} values was 0.031 and 0.23 g/L against *B. cinerea* and *P. grisea*, respectively.

Thiosemicarbazones was well documented for its antibacterial, antifungal, antiviral, antitumor, and antimalarial activities (Beraldo & Gambinob, 2004; Zhong, Aotegen, & Xu, 2011). The biological properties of thiosemicarbazones have aroused considerable interest in the past few years. The present work was to combine thiosemicarbazones with chitosan, to prepare more potent antifungal chitosan derivatives, and to assess their antifungal activity against common crop-threatening pathogenic fungi.

2. Experiment

2.1. Materials

Chitosan, 87% degree of deacetylation and average-molecular weight of 230 kDa, was purchased from Qingdao Baicheng Biochemical Corp. Other chemical reagents were all analytical grade. Polyoxin, 10% wettable powders, was purchased from Kaken Pharmaceutical Co., Ltd. Four crop-threatening pathogenic fungi (*S. solani*, *R. solani*, *A. solani*, and *P. asparagi*) were obtained from Qingdao Academy of Agricultural Sciences.

2.2. Analytical methods

Fourier transform infrared (FT-IR) spectra of the derivatives were measured in the 4000–400 cm^{-1} regions using a Thermo Scientific Nicolet iS10 FT-IR spectrometer in KBr discs.

Solid-state ^{13}C CP/MAS NMR spectra were obtained from a Bruker AC-300 spectrometer. A ramped contact-power cross-polarisation (CP) pulse sequence was applied and a double-air-bearing Magic Angle Spinning (MAS) probe with 7 mm external diameter sample rotors was employed. Time domain size is 2048. The number of scans is 1000.

The elemental analysis (C, H, N, and S) was performed on a Vario EL-III elemental analyzer. The percentages of carbon, hydrogen, nitrogen, and sulfur were estimated.

XRD measurement of the powder samples were performed with a D8 advance diffractometer (Bruker) with Cu target ($\lambda = 0.154$ nm) at 40 kV. The scanning rate was 1.2°/min and the scanning scope of 2θ was 5–50°.

DSC temperature scan was performed using the Pyris Diamond DSC (Perkin Elmer). The samples were putted into aluminum cup and sealed. An empty pan was used as reference in the test. The samples were heated from 50 °C to 250 °C under 10 °C/min. Nitrogen gas was used in the experimental work to confirm thermal behavior.

2.3. Synthesis of thiosemicarbazone chitosan (TCNCS) derivatives

2.3.1. One pot synthesis of thiosemicarbazide chitosan (TCDCS)

Thiosemicarbazide chitosan were prepared via one pot synthesis, in which preparation of ammonium dithiocarbamate chitosan was according to the method in the literature with minor revision (Muzzarelli, Tanfani, Mariotti, & Emanuelli, 1982). A mixture of chitosan (16 g) and ammonium hydroxide (20 mL) was stirred in 95% ethanol for half an hour at room temperature. Then carbon disulfide (8 mL) was slowly dropped into the mixture. After stirring for 2 h, ammonium dithiocarbamate chitosan (1) was obtained. Next, sodium chloroacetate (11.5 g) was added into ammonium dithiocarbamate chitosan (1) and keep the mixture reacting for 30 min to get sodium carbethoxy dithiocarbamate chitosan (2). At last, 85% hydrazine hydrate (12 mL) was slowly added to (2) at room temperature. After reacted for another 2 h, the resulting mixture was filtered through Filter Funnel Buchner. The residue was washed with ethanol and dried at 60 °C. Thiosemicarbazide chitosan (TCDCS), a light brown powder, was obtained (Scheme 1).

2.3.2. Preparation of thiosemicarbazone chitosan (TCNCS) derivatives

Thiosemicarbazide chitosan (10 mmol) mixed with phenylaldehyde (o-hydroxyphenylaldehyde, p-methoxyphenylaldehyde) (10 mmol) in methanol (100 mL). Then acetic acid (0.2 mL) was added slowly. After refluxed for 10 h, the resultant was cooled to room temperature and filtered through Filter Funnel Buchner. The precipitate was washed with methanol and dried at 60 °C to give phenylaldehyde thiosemicarbazone chitosan (PHTCNCS), o-hydroxyphenylaldehyde thiosemicarbazone chitosan (o-HPHTCNCS), and p-methoxyphenylaldehyde thiosemicarbazone chitosan (p-MOPHTCNCS) derivative (Scheme 1).

2.4. Antifungal assay

Antifungal assays were evaluated in vitro by mycelium growth rate test (Hernández-Lauzardo et al., 2008). Chitosan and thiosemicarbazone chitosan derivatives were dissolved in 1% (v/v) acetic acid at an original concentration of 2% (w/v). The solutions were autoclaved at 121 °C for 20 min and mixed with sterile molten potato dextrose agar (PDA) to obtain final concentrations of 0.05 mg/mL, 0.1 mg/mL, and 0.5 mg/mL. When the agar was cooled, the mycelium of fungi was transferred to the plates and then incubated at 27 °C. The mixed medium without sample was used as the blank control. When the mycelium of fungi reached the edges of the blank control plate, the antifungal index was calculated with the following equation:

$$\text{Antifungal index}(\%) = \frac{D_b - D_t}{D_b} \times 100$$

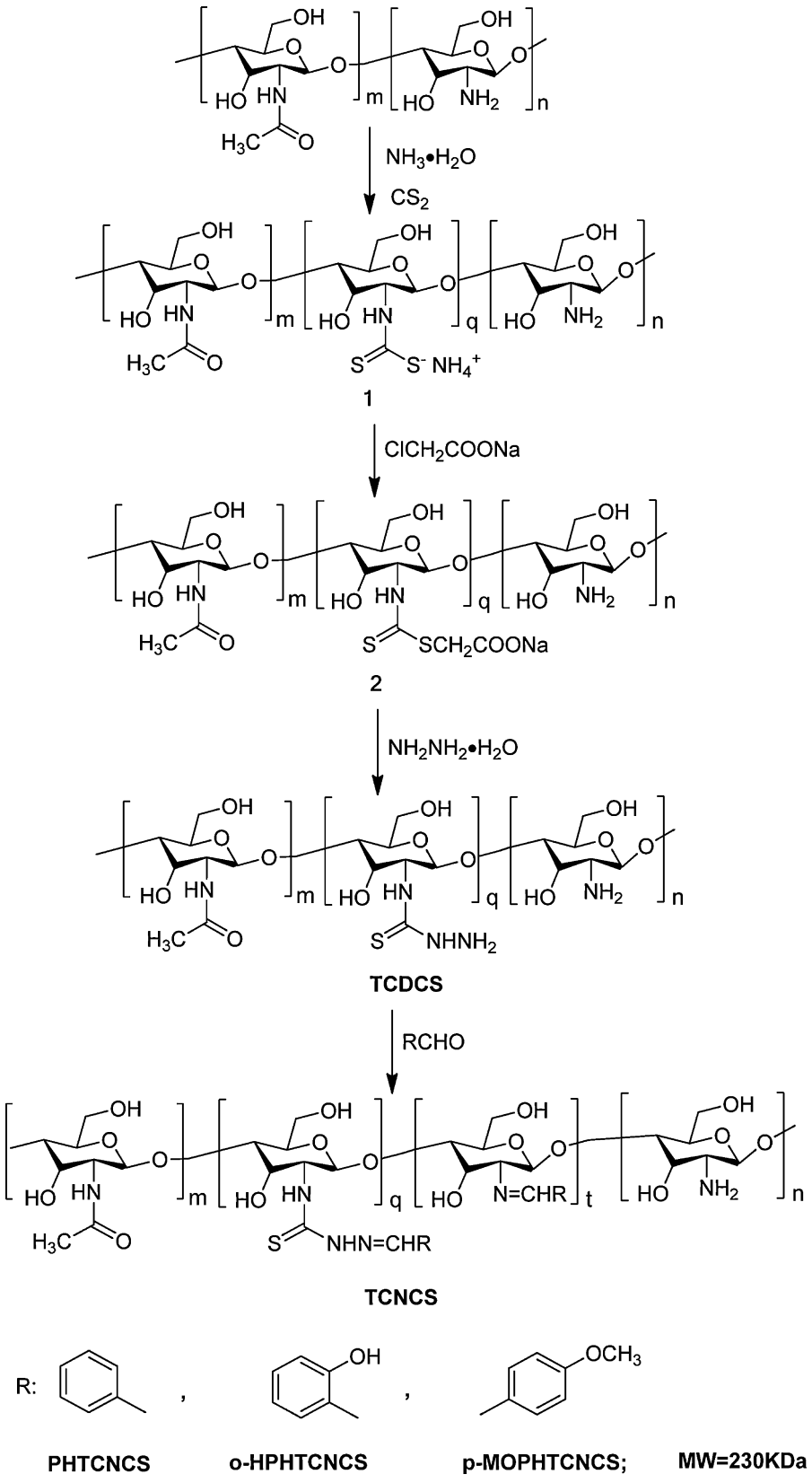
Here, D_t is colony diameter in the test plate and D_b is colony diameter in the blank control.

Three replicates of each test were carried out and the results were averaged. The Scheffe method was used to evaluate the differences in antifungal index in the tests. Results with $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Preparation and characterization of thiosemicarbazone chitosan (TCNCS) derivatives

Condensation reaction of thiosemicarbazide with aldehydes or ketones is the general method for preparation of thiosemicarbazone (Cunha & da Silva, 2009). So in the synthetic procedures of thiosemicarbazone chitosan, thiosemicarbazide chitosan (TCDCS) was the key intermediate. Available methods for synthesis of thiosemicarbazide include: (a) hydrazinolysis of carbamodithioate (Kovala-Demertzi et al., 2009); (b) hydrazine react with isothiocyanate (Ghosh, Misra, Bhatia, Khan, & Khanna, 2009); (c) hydrazinecarbodithioate react with amine (Krishnan et al., 2008). To prepare thiosemicarbazone chitosan (TCNCS), route (a) is feasible and mild. For route (b), it is difficult to obtain hydrazine chitosan or isothiocyanate chitosan. Route (c) is also a feasible scheme to prepare thiosemicarbazide chitosan. However, this method for preparation of thiosemicarbazide chitosan has been reported in the literature (Zhong, Zhong, Xing, Li, & Mo, 2010). So in this study route (a) was chosen to synthesize thiosemicarbazide chitosan.



Scheme 1. Synthesis of thiosemicarbazide chitosan (TCDCS) and thiosemicarbazone chitosan (TCNCS) derivatives.

As shown in Scheme 1, thiosemicarbazide chitosan was one pot synthesized by mixing chitosan with carbon disulphide, sodium chloroacetate, and hydrazine hydrate. The approaches avoided isolation and purification of each prepared intermediate, and save time, solvent, and energy consuming procedures. In contrast, we noticed that the free amino group at C-2 position in chitosan was not fully substituted by thiosemicarbazide group due to poor solubility of chitosan in ethanol. Hence, the structures of TCNCS also was

partly a Schiff base because of the reaction between some primary amino groups with the aldehydes.

Fig. 1 showed FT-IR spectra of CS, TCDCS, PHTCNCS, o-HPHTCNCS, and p-MOPHTCNCS. The broad band ranged from 3300 cm^{-1} to 3440 cm^{-1} attributed to –OH and –NH stretching vibration. The weak band at 2875 cm^{-1} assigned to the characteristic absorbance peak of –CH. The absorption peak at 1598 cm^{-1} was for NH_2 bending vibration. Additionally, the absorption peaks at 1155 cm^{-1}, 1075 cm^{-1} and 1025 cm^{-1} attributed to asymmetric stretching of the C–O–C stretching, the skeletal vibration. Compared with CS, TCDCS showed appearance of a new band at 1550 cm^{-1}, which is attributed to the –NH–CS–NH– group. In addition, the signal from amide I at 1645 cm^{-1} (C=O) disappeared, the peak at 1598 cm^{-1} of the NH bending in the primary amine became weak which meant part of amino has been substituted (Zhong et al., 2010). A new peak at 1650 cm^{-1} (–NH–NH_2) was also observed. All of the above results showed TCDCS had been successfully obtained.

Compared with TCDCS, the new bands at 1642 cm^{-1} (–C=N–), 761 cm^{-1} (phenyl), 694 cm^{-1} (phenyl) showed PHTCNCS had been obtained. Likewise, new bands at 1634 cm^{-1} (–C=N–), 758 cm^{-1} (phenyl) indicated o-HPHTCNCS had been achieved; the absorption bands at 1648 cm^{-1} (–C=N–), 836 cm^{-1} (phenyl) can confirm the chemical structures of p-MOPHTCNCS.

The solid-state CP-MAS ^{13}C NMR technique has been used to characterize structures of chitosan (De Angelis, Capitani, & Crescenzi, 1998). In order to further confirm structures of the chitosan derivatives, solid-state CP-MAS ^{13}C NMR was performed. The ^{13}C NMR spectrum of chitosan was shown in Fig. 2(a). Spectra of chitosan coincided with that reported before (de Britto & Assis, 2007). The following signals can be identified: (1) δ = 25 ppm represented the carbon of the methyl; (2) δ = 57 ppm and δ = 63 ppm, the two signals attributed to carbon C2 and C6 respectively; (3) δ = 78 ppm represented carbon C5 and C3; (4) δ = 84 ppm attributed to carbon C4; (5) δ = 106 ppm attributed to carbon C1; (6) δ = 178 ppm attributed to carbon C=O, meaning the chitosan sample not fully deacetylated. Compared with CS, it was obvious that a new weak signal for substituted C2 appeared at 67 ppm (C2′). This indicated some of amino at C2 were substituted. The signal at 63 ppm was still observed. It meant lots of amino at C2 did not react. This result coincided with that of FT-IR above. Additionally, the peak at 178 ppm became broad. It may be caused by C=S group, of which signal was superimposed by the signals for C=O (Chen, Wu, & Zeng, 2005). Hence, we can conclude that TCDCS was obtained.

Table 1
Elemental analysis results and degree of substitution of thiosemicarbazone chitosan (TCNCS) derivatives.

Sample	Elemental analysis (%)				Degree of substitution (%)
	C	N	H	S	
CS	40.05	7.29	6.41	–	–
TCDCS	37.63	7.02	6.26	1.52	11.20
PHTCNCS	49.81	6.06	5.64	0.93	9.42
o-HPHTCNCS	50.99	5.94	5.37	0.81	8.62
p-MOPHTCNCS	47.11	5.79	5.82	1.02	11.20

Compared with TCDCS, new peaks for PHTCNCS at 125–140 ppm attributed to carbon of phenyl; δ = 170 ppm attributed to carbon N=CH– group. These showed PHTCNCS had been prepared. Similarly, δ = 165 ppm (N=CH–), δ = 138 ppm (Ph–H), δ = 125 ppm (Ph–H) confirmed the structures of o-HPHTCNCS; δ = 167 ppm (N=CH–), δ = 130 ppm (Ph–H) meant p-MOPHTCNCS to be obtained.

Table 1 showed elemental analysis results. The degree of substitution (DS) of thiosemicarbazone chitosan derivatives was calculated on the basis of the percentage. As shown in Table 1, the DS of PHTCNCS, o-HPHTCNCS, and p-MOPHTCNCS was 9.42%, 8.62%, 11.20%, respectively. The DS of the chitosan derivatives was not so high. It meant only few thiosemicarbazone groups were introduced into chitosan. This also accorded with above results. And we can infer that Schiff base group was also incorporated with chitosan.

The XRD spectra of chitosan, thiosemicarbazide chitosan (TCDCS), and thiosemicarbazone chitosan derivatives (TCNCS) were shown in Fig. 3. The characteristic peaks of chitosan appeared at 2θ = 10.7° and 20.1°. The reflection at 2θ = 10.7° was assigned to crystal forms I. The strongest peak appeared at 2θ = 20.1° attributed to crystal forms II (Wu et al., 2005). Spectrum of thiosemicarbazide chitosan (TCDCS) was similar to that of chitosan. However, there

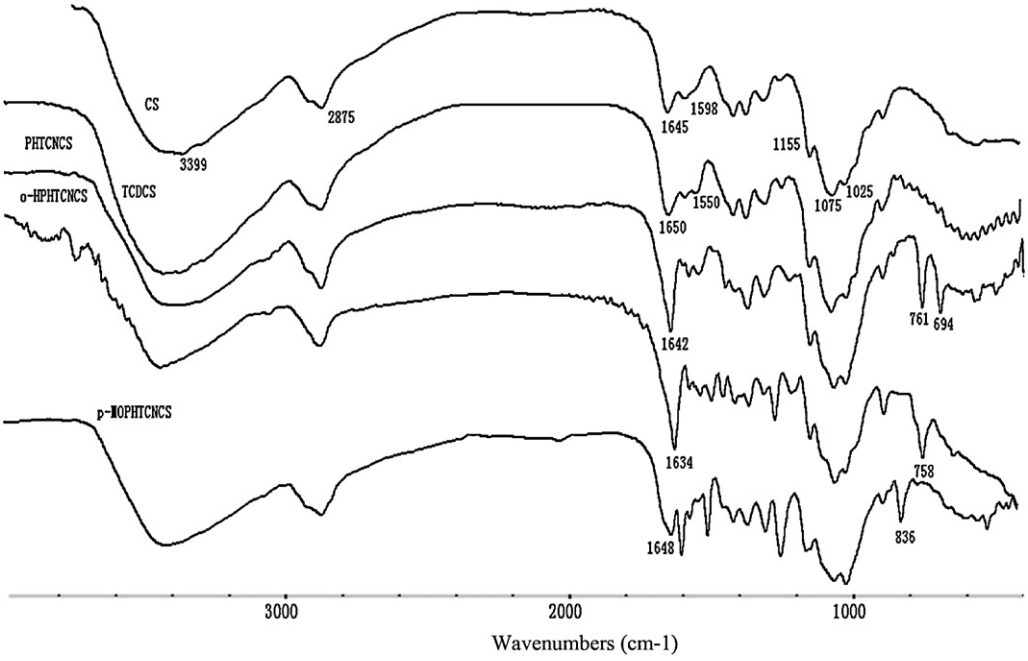


Fig. 1. FT-IR spectra of chitosan, thiosemicarbazide chitosan (TCDCS), and thiosemicarbazone chitosan (TCNCS) derivatives.

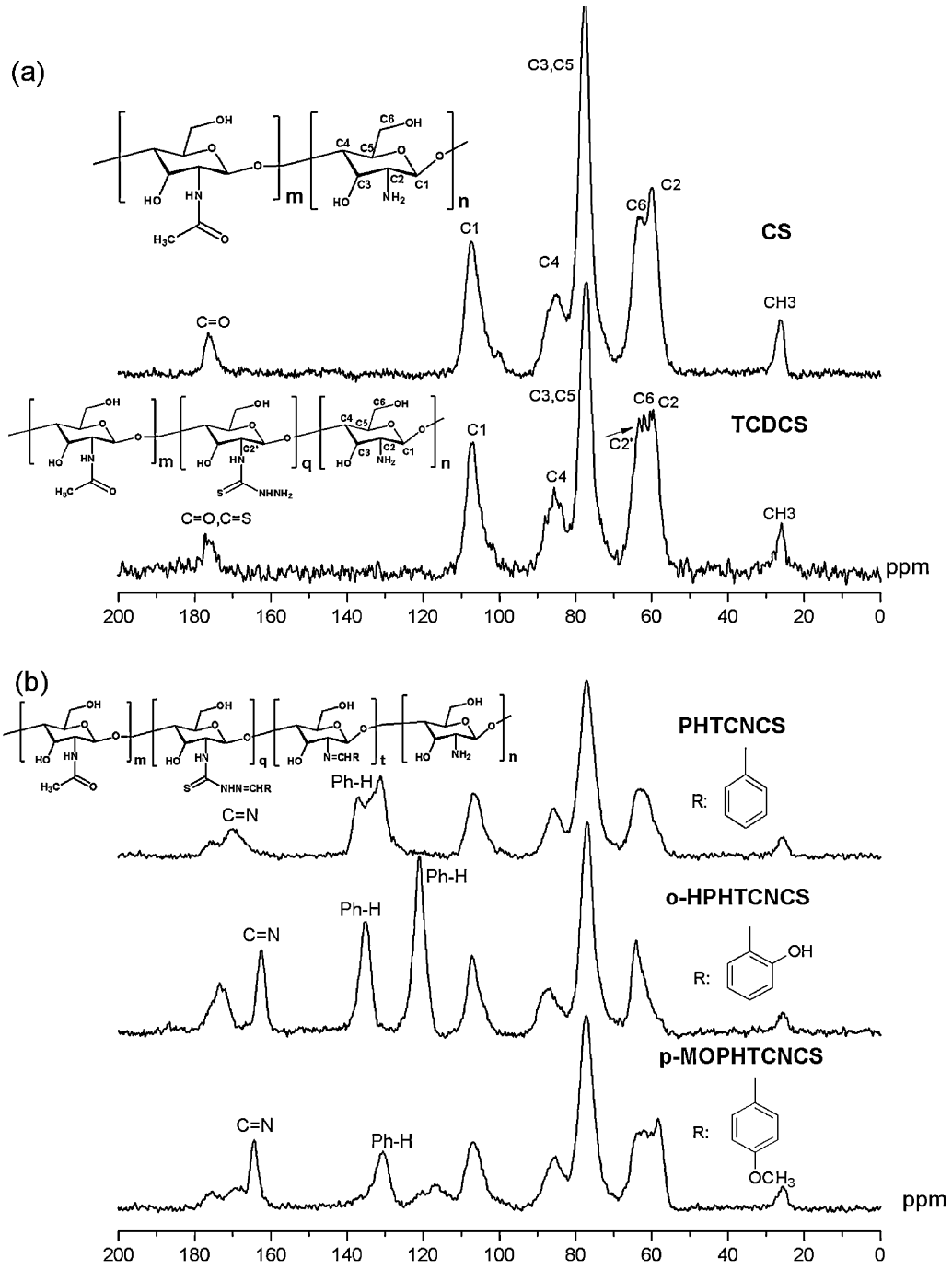


Fig. 2. (a) Solid state CP-MAS ^{13}C NMR spectra of chitosan and thiosemicarbazide chitosan (TCDCS) and (b) ^{13}C NMR spectra of thiosemicarbazone chitosan (TCNCS) derivatives.

was still little difference between them. A new peak of TCDCS exhibited at $2\theta = 10.2^{\circ}$. It indicated the crystalline structure of chitosan slightly changed via chemical modification. Meanwhile, it also suggested that the original crystallinity of chitosan was not fully destroyed. This also coincided with above results.

Compared with CS and TCDCS, new sharp and high-intensive peaks for TCNCS (PHTCNCS, o-HPHTCNCS, and p-MOPHTCNCS) appeared at 6.7°, 6.4°, and 6.2°, respectively. It suggested that the presence of thiosemicarbazone group formed a highly ordered structure. The crystalline structure transformed from amorphous structure in to a relatively crystalline structure in chitosan and TCDCS to TCNCS derivatives (Hu et al., 2007; Singh et al., 2009).

DSC heating profiles of chitosan and TCDCS were shown in Fig. 4. All the samples showed an broad endothermic peak around 80–160 °C. These may result from evaporation of water present in the samples (Kittur, Prashanth, Sankar, & Tharanathan, 2002).

Polymers such as polysaccharides are normally found with high hydrophilic capacity. The hydration properties relate to the primary and super molecular structures (Phillips, Takigami, & Takigami, 1996). Moisture influences both crystalline and amorphous phases of the structure. Therefore, the endothermic peak corresponding to the evaporation of water was expected to reflect physical and molecular changes during chemical modification. Fig. 4 showed the endothermic peak area increased after the modification of chitosan; the endothermic peak value of the derivatives decreased compared with chitosan, thus indicating that the crystal structure of chitosan was partly changed as a consequence of the formation of a chitosan derivative.

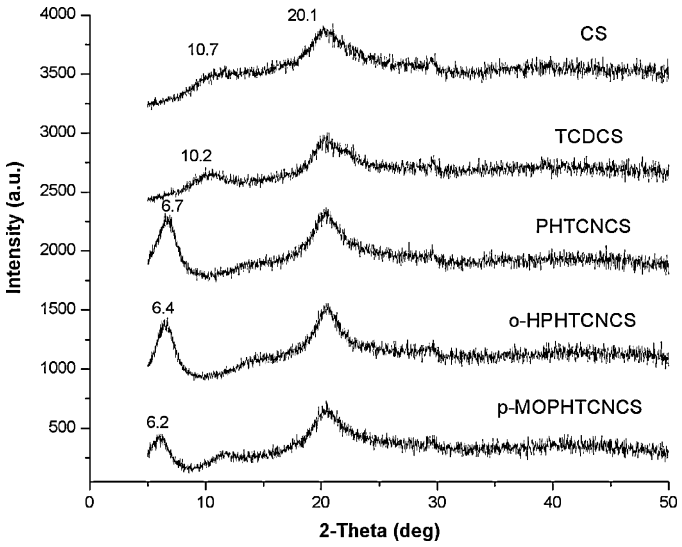


Fig. 3. X-ray diffraction patterns of chitosan, thiosemicarbazide chitosan (TCDCS), and thiosemicarbazone chitosan (TCNCS) derivatives.

3.2. Antifungal activity of thiosemicarbazone chitosan (TCNCS)

The antifungal results of the chitosan derivatives against the fungi were shown in Table 2. In general, all the derivatives showed significant antifungal activity against the fungi especially *S. solani* and *R. solani*.

As shown in Table 2, all the derivatives exhibited much stronger inhibitory effect on *S. solani* than original chitosan, and the antifungal activity increased with increasing concentration. Antifungal index of the derivatives ranged from 21.6% to 64.9%, while the index of chitosan ranged from 0% to 38.5%. The inhibitory effect followed a sequence of o-HPHTCNCS > p-MOPHTCNCS > PHTCNCS. It was inferred various substituents of aromatic ring in chitosan derivatives had distinct impacts on antifungal activity. In addition, antifungal activity of o-HPHTCNCS against *S. solani* was even equal to that of polyoxin. It was concluded that antifungal activity of chitosan enhanced obviously via chemical modification. The introduction of the substituent group at C-2 position increased the antifungal activity of CS. There were two kinds of substituent groups (thiosemicarbazone and Schiff base) at C-2 position in chitosan. Our former study showed that Schiff base chitosan derivatives had weaker antifungal activity than chitosan. For example, Guo et al. (2007b) reported the Schiff bases of chitosan (As and Bs) have a slight activity against *B. cinerea Pers.* The inhibitory indices of them at 1000 ppm were 26.8% and 33.5%, while the inhibitory index of chitosan was 45.4%. Guo et al. (2006) also prepared the Schiff bases of carboxymethyl chitosan (CMCTS) and found 2-(hydroxybenzylideneamino)-6-carboxymethylchitosan (HCMCTS) had lower antifungal activity than original chitosan. Based on the former study, we inferred antifungal group of the derivatives was thiosemicarbazone but not Schiff base. Considering that so low DS (about 10%) of TCNCS leaded to a great improvement on antifungal activity of CS, further study should be continued to enhance the degree of TCNCS and evaluate effect of the substituent group on antifungal activity of CS.

Table 2
Antifungal activity of thiosemicarbazone chitosan (TCNCS) derivatives at different concentrations.

Sample	Concentrations (mg/mL)	*S. solani*	*R. solani*	*A. solani*	*P. asparagi*
CS	0.05	0	10.3	5.5	0
	0.1	0	12.5	16.6	27.0
	0.5	38.5	53.4	88.9	50.0
PHTCNCS	0.05	21.6	38.4	13.9	5.3
	0.1	24.3	52.6	16.7	12.0
	0.5	64.9	97.0	84.5	49.7
o-HPHTCNCS	0.05	30.4	52.6	1.6	6.0
	0.1	43.2	60.3	19.0	18.7
	0.5	62.2	100	90.1	68.3
p-MOPHTCNCS	0.05	35.1	26.7	12.7	7.0
	0.1	38.5	36.2	19.0	11.3
	0.5	52.0	100	91.7	55.7
Polyoxin	0.05	31.1	31.5	27.4	27.0
	0.1	39.9	62.9	33.7	39.7
	0.5	66.2	100	93.2	98.3

As shown in Table 2 and Fig. 5, the inhibitory index of the derivatives against *R. solani* ranged from 26.7% to 100%. Similar to *S. solani*, the antifungal activity also followed o-HPHTCNCS > p-MOPHTCNCS > PHTCNCS. At lower concentration of 0.05 mg/mL, o-HPHTCNCS 52.6% inhibited growth of the mycelium, stronger than polyoxin whose antifungal index was 31.5%. For o-HPHTCNCS, p-MOPHTCNCS, and PHTCNCS, the inhibitory index at 0.5 mg/mL was 100%, 100%, and 97.0% respectively, much higher than that of chitosan.

Antifungal activity of the derivatives against *A. solani* was shown in Table 2. The inhibitory index of the derivatives ranged from 1.6% to 91.7%. Compared with CS, PHTCNCS, o-HPHTCNCS, and p-MOPHTCNCS only exhibited slightly better antifungal activity against the *A. solani*. It meant introduction of the substituent group

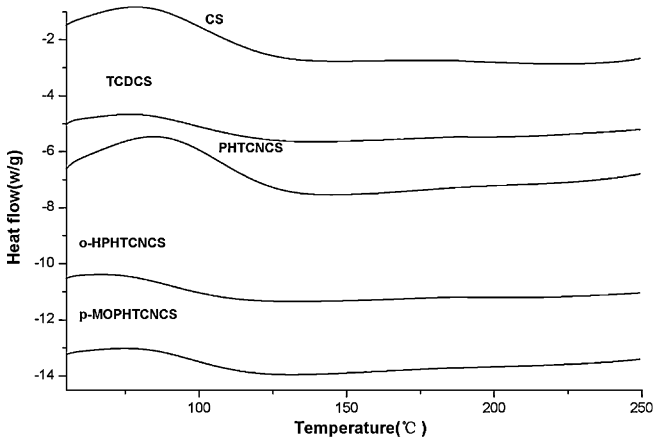


Fig. 4. DSC curves of chitosan, thiosemicarbazide chitosan (TCDCS), and thiosemicarbazone chitosan (TCNCS) derivatives.

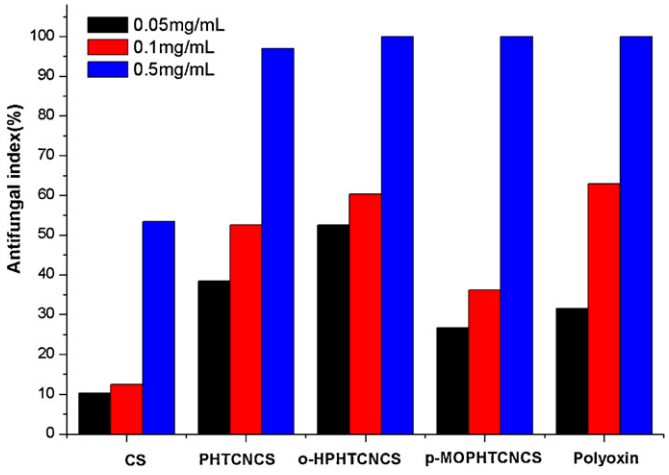


Fig. 5. Antifungal activity of thiosemicarbazone chitosan (TCNCS) derivatives against *R. solani*.

at C-2 position did not obviously increase the antifungal activity of CS against the fungus. It was deduced the fungus was not so sensitive as *S. solani* and *R. solani* to TCNCS.

Table 2 presented antifungal result of the chitosan derivatives against *P. asparagi*. Antifungal activity was influenced by concentration of the derivatives. The higher the concentration was, the stronger the antifungal activity was. At low concentration of 0.05 mg/mL, all the derivatives showed weak antifungal activity. And at high concentration of 0.5 mg/mL, the inhibitory index of them was not less than 49.7%. However, their antifungal activity was not so strong as polyoxin.

From the above bioassay results, it was concluded that thiosemicarbazone chitosan derivatives inhibited the fungi more effectively than chitosan. The antifungal activity depended on concentration of the derivatives and species of the fungi.

4. Conclusion

Three novel structural thiosemicarbazone chitosan derivatives were prepared. Antifungal activity against *S. solani*, *R. solani*, *A. solani*, and *P. asparagi* showed that the thiosemicarbazone chitosan derivatives greatly inhibited the fungi. All of them had broad-spectrum antifungal activity that depended on concentration of the derivatives and fungal species. *S. solani* and *R. solani* were more sensitive than *A. solani* and *P. asparagi*. The inhibitory effect followed the sequence: o-HPHTCNCS > p-MOPHTCNCS > PHTCNCS. Additionally, antifungal activity of o-HPHTCNCS against *S. solani* and *R. solani* was comparable to that of polyoxin. The preliminary structure-activity relationship showed that: the antifungal group of the derivatives was thiosemicarbazone but not Schiff base; various substituents of aromatic ring in chitosan derivatives had distinct impacts on antifungal activity. Although the DS of the derivatives was not so high (ca. 10%) their antifungal activity was significantly higher than that of chitosan and chitosan Schiff bases. These data are of importance because they target common crop-threatening pathogenic fungi.

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