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Fungicidal and Insecticidal Activity of *O*-Acyl Chitosan Derivatives

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Summary

A series of *O*-(acyl) chitosan (OAC) derivatives with a degree of substitution (DS) between 0.02 and 0.28 were synthesized by reaction of alkanoic acid derivatives with chitosan in the presence of H_2SO_4 as a catalyst. The reaction was performed at 80°C for 4h with different mol ratios of alkanoic acids to chitosan. The synthesized compounds were analyzed by ^{1}H- and ^{13}C-NMR spectroscopy. A high DS was obtained with *O*-(butyroyl) chitosan (DS 0.28) at a mol ratio of (1:5) chitosan to butyric acid. Their fungicidal activity against the grey mould *Botrytis cinerea* (Leotiales: Sclerotiniaceae) and the rice leaf blast pathogen *Pyricularia grisea* (Teleomorph: Magnaportha grisea) has been evaluated. *O*-(decanoyl) chitosan at mol ratio of 1:2 (chitosan to decanoic acid) was the most active compound against *B. cinerea* (EC_{50} = 1.02 g.l^{-1}) and *O*-(hexanoyl) chitosan displayed the highest activity against *P. grisea* (EC_{50} = 1.11 g.l^{-1}). It has been mentioned that some derivatives also repressed spore formation at rather high concentrations (1.0, 2.0 and 5.0 g.l^{-1}). The insecticidal activity has been screened at 5 g.kg^{-1} artificial diet against the larvae of the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae). The results revealed that all of the synthesized derivatives showed high inhibition of growth of the larvae of *S. littoralis* compared to chitosan (7% growth inhibition) and the most active one was *O*-(decanoyl) chitosan (64% growth inhibition) after 5 days of feeding on treated artificial diet.

Introduction

Chitosan, a biomaterial obtained by alkaline deacetylation of natural chitin, has recently attracted much attention from scientists in different parts of the world [1-3]. It consists of β-1,4-linked 2-amino-2-deoxy-D-glucopyranose units and has been found to be a useful functional material [4,5] and offers a special set of characteristics: biocompatibility, biodegradability, and anti-bacterial properties [6,7]. It is also safe for human use, and quite stable in the natural environment [5,8,9]. The above characteristics make chitosan suitable for use in a number of biomedical, biological and industrial applications. Therefore, special attention was paid to its chemical modification and several chitosan derivatives have been prepared such as

O-, *N*-(carboxymethyl) chitosan [6], *N*-(carboxymethyl) chitosan [7], *O*-(carboxymethyl) chitosan [10,11], *N*-(carboxyethyl) chitosan [12,13], *N*-(sulphate) chitosan [14], *O*-(sulphate) chitosan [15], *O*-(butyroyl) chitosan [16], *N*-(methylenephosphonic) chitosan [17,18], *N*-(benzylphosphoryl) chitosan [19], hydroxypropyl chitosan [20], *N*-(trimethyl) chitosan [21], *N*-(succinyl) chitosan [22], *O*-(succinyl) chitosan [23], *N*-(alkyl) and *N*-(benzyl) chitosans [24] and *N,O*-(acyl) chitosans [25].

The application of chitosan in agriculture is recently becoming a major focus of research. Research has shown that chitosan and its derivatives can be used as plant growth regulators, fungicides, and seed coating agents by the induction of phytoalexins [24-28]. Chitosan has been shown to be fungicidal against several plant pathogenic fungi including *Botrytis cinerea* [29] and the minimum inhibitory concentrations (MIC) reported for specific target organisms range from 10 to 5000 ppm and are influenced by a multitude of factors such as the pH of the growth medium, the degree of polymerization of chitosan, and the presence or absence of interfering substances such as lipids and proteins. Recently the fungicidal activity of chitosan derivatives, including *N*-alkyl, *N*-benzyl and *N,O*-acyl chitosans showed strong biological activity against the plant pathogenic fungi *B. cinerea* and *Pyricularia grisea* compared to the native chitosan.

In addition, *N*-(alkyl), *N*-(benzyl) and *N,O*-(acyl) chitosans were demonstrated insecticidal action against the cotton leafworm *Spodoptera littoralis* [26-28]. Chitosan was also tested against *Helicoverpa armigera*, *Plutella xylostella*, and aphids (*Aphis gossypii* (Glover), *Metopolophium dirhodum* (Walker), *Hyalopterus prun* (Goffroy) *Rhopalosiphum padi, Sitobion avenae* (Fabricius) and *Myzus persicae* (Sulzer)) [30-34].

In the present article, the synthesis of a series of *O*-(acyl) chitosan (OAC) derivatives and evaluation of their fungicidal activity against the grey mould *B. cinerea* (Leotiales: Sclerotiniaceae) and the rice leaf blast pathogen *Pyricularia grisea* is reported. Also the insecticidal and growth-inhibitory activities against larvae of the cotton leafworm *S. littoralis*, a polyphagous insect pest of world importance in cotton, vegetables and ornamentals are discussed.

Experimental Section

Materials

Chitosan of low molecular weight (made from coarse ground crab) and all chemicals were purchased from Sigma-Aldrich Co. (Bornem, Belgium). All materials were used without further purification. For the fungicidal bioassay, Potato Dextrose Agar (PDA) was purchased from Oxoid Ltd. (Basingstoke, Hampshire, England) and Oatmeal Agar (OMA) from Becton Dickinson France S.A. (Le Pont de Clair, France). For the insect bioassay, soybean-wheat germ insect artificial diet was purchased from Stonefly Ind. (Bryan, TX, USA).

Methods

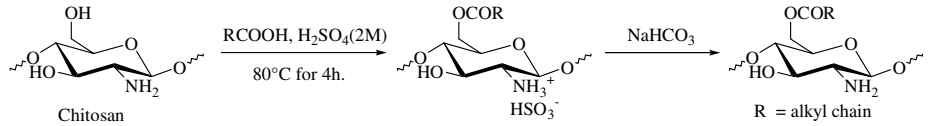
NMR spectroscopy

^{1}H- and ^{13}C-NMR measurements were performed on a JEOL A-300 NMR spectrometer under a static magnetic field of 300 MHz, at 25°C or 70°C. For those measurements, 20 mg of sample was introduced into 5 mm Φ NMR tube, to which

0.5 ml of 1% CF_3COOD/D_2O solution was added, and finally the tube was kept at 70°C to dissolve the polymer.

Synthesis of OAC derivatives

OAC derivatives were synthesized as follows (Scheme 1): 18 mmol of chitosan (3 g calculated as glucosamine units) was suspended in 100 ml of distilled water. To this solution the acid derivative (1-5 equivalent/glucosamine unit of chitosan) was added. Then, 5 ml of H_2SO_4 (2M) was added dropwise at room temperature. The mixture was stirred at 80°C for 4h and was cooled subsequently to room temperature. The pH was adjusted to 7 by neutralization with $NaHCO_3$. The desired compound was precipitated in acetone, filtered and washed with acetone to remove the unreacted acid. Finally, the precipitants were soxhlet-extracted with acetone for 2 days and then oven-dried overnight at 60°C, giving the titled compounds.



Scheme 1. Synthetic route to OAC derivatives.

Fungicidal bioassay

Chitosan and OAC derivatives solutions were prepared by dissolving these compounds in aqueous 1% trifluoroacetic acid (TFA), and the pH was adjusted to 5.5-6.0 with 1M NaOH [25,35]. PDA and OMA media for *B. cinerea* and *P. grisea*, respectively, containing different concentrations of chitosan or the synthesized derivatives ranging between 0 and 6.0 $g.L^{-1}$ were seeded in sterile culture plates (9-cm diameter) and infected with 6-mm-diameter agar plugs taken from the margin of a 7-day old culture of *B. cinerea* or a 10-day old culture of *P. grisea*. For each compound, 4 replicates were used for each fungus per concentration tested. The plates were incubated in the dark at 26 ± 2°C for *B. cinerea* or 30 ± 2°C for *P. grisea* [36]. Growth measurements were determined when the hyphae in the control had grown up to the edge of the plate which was after 7 days for *B. cinerea* and 10 days for *P. grisea*. EC_{50}'s and corresponding 95% CL were estimated by probit analysis [37].

Insecticidal and growth inhibitory bioassay

In a standardized screening toxicity test, third-instar larvae of *S. littoralis* were selected from a laboratory colony reared on artificial diet under controlled conditions at 25±2°C, 70±5% RH and a 16h light photoperiod [38,39]. Chitosan and synthesized derivatives were dissolved in aqueous 1% TFA and tested at 5 $g.kg^{-1}$ in artificial diet, with 30 larvae per product each. Growth inhibitory effect was scored during the experiment until the 5^{th} day and the mortality was scored on the 7^{th} day of the treatment and compared with the control that had been exposed to a diet treated with solvent only. The growth inhibition was calculated from this equation:

$$\text{Growth inhibition } (\%) = [(C_L - T_L)/C_L]*100$$

where C_L is the larval weight gained in the control and T_L is the larval weight gained in the treatment.

Results and Discussion

Synthesis of OAC derivatives

The reaction of chitosan with different mol ratios of alkanoic acids per glucosamine residue was carried out at 80°C for 4h (Table 1). The chemical structures of chitosan and OAC derivatives were confirmed by ^{1}H and ^{13}C-NMR spectroscopy (Table 2). The molecular fraction (MF) of each functional group was estimated by ^{1}H-NMR. The results in Table 1 indicated that the reaction mainly occurred on the OH group and not on the NH_2 group. This is shown in Table 1 as MF of NH_2, which is almost the same as the original chitosan.

All DS values are < 1. This indicates that the reaction mainly occurred at the one OH group (at C-3 or C-6) of the chitosan molecule. It is very clear that exchange of hexanoic acid to 6-aminohexanoic acid leads to a high increase of the DS value (see compounds 11 and 12 vs. 14 and 15). The synthesized derivatives are well soluble in an aqueous 1% TFA solution.

Table 1. Chemical structure and properties of OAC derivatives.

Entry	R	Mol ratio (chitosan/acid)	MF of NH_2[a] (x)	DA[b] (y)	DS[c] (z)	FW[d]
Chitosan	-	-	0.89	0.11	-	166
1	CH_3CH_2	1:3	0.90	0.11	0.09	172
2	$CH_3(CH_2)_2$	1:3	0.88	0.11	0.16	175
3	$CH_3(CH_2)_2$	1:5	0.86	0.14	0.28	187
4	$CH_3CH_2CH(CH_3)$	1:3	0.88	0.12	0.07	172
5	$CH_3(CH_2)_3$	1:3	0.82	0.16	0.10	173
6	$CH_3(CH_2)_3$	1:5	0.91	0.11	0.14	181
7	$ClCH_2(CH_2)_3$	1:3	0.87	0.12	0.05	170
8	$ClCH_2(CH_2)_3$	1:5	0.91	0.08	0.13	178
9	$BrCH_2(CH_2)_3$	1:1	0.91	0.11	0.04	175
10	$ClCH_2C(CH_3)_2$	1:3	0.89	0.12	0.03	171
11	$CH_3(CH_2)_4$	1:3	0.90	0.11	0.02	169
12	$CH_3(CH_2)_4$	1:5	0.89	0.12	0.03	171
13	$CH_3CH=CHCH=CH$	1:1	0.90	0.10	0.04	169
14	$H_2N(CH_2)_5$	1:1	0.88	0.11	0.20	187
15	$H_2N(CH_2)_5$	1:2	0.90	0.10	0.22	190
16	$CH_3(CH_2)_5$	1:3	0.88	0.12	0.10	177
17	$CH_3(CH_2)_5$	1:5	0.87	0.12	0.23	190
18	$CH_3(CH_2)_6$	1:3	0.91	0.12	0.02	173
19	$CH_3(CH_2)_6$	1:5	0.91	0.11	0.04	174
20	$CH_3(CH_2)_7$	1:3	0.91	0.11	0.04	174
21	$CH_3(CH_2)_7$	1:5	0.87	0.13	0.16	189
22	$CH_3(CH_2)_8$	1:1	0.89	0.12	0.05	175
23	$CH_3(CH_2)_8$	1:2	0.91	0.10	0.09	181

[a] MF (NH_2, X) was calculated from this equation: B/C = X/(6-X),
[b] DA or MF of (NHAc, y) calculated from: A/(B+C) = 3DA/6,
[c] DS (degree of substitution, z) calculated from D/(B+C) = nDS/6
Where: **A** is the peak area of NHAc, **B** is the peak area of H-2 of GlcN unit, **C** is the peak area of H-2 of GlcNAc and H-3,4,5,6 of GlcN; **D** is the peak area of substituent and **n** is the number of hydrogen atom per substituent.
[d] FW = (161 X MF of NH_2 + 203 X DA + DS × MW of substituent).

Table 2. Spectral data of OAC derivatives.

Entry	^{1}H-NMR (δ ppm)	^{13}C-NMR (δ ppm)
Chitosan	2.1 (s, NHAc), 3.2 (t, H-2 of GlcN), 3.5-4.1 (br m, H-2 of GlcNAc and H-3,4,5,6 of GlcN), 4.6 (br s, H-1 of GlcNAc), 4.9 (d, H-1 of GlcN)	22.08 ($\underline{C}H_3$), 56.6 (C-2), 61.2 (C-6), 70.6 (C-3), 75.4 (C-5), 77.7 (C-4), 98.2 (C-1), 174.8 ($\underline{C}(O)CH_3$)
1	1.05 (t, CH_3), 2.36 (q, CH_2 (α))	-
2	0.9 (t, CH_3), 1.5 (q, CH_2 (β)), 2.3 (q, CH_2 (α))	11.7 (CH_3), 17.1 (CH_2 (β)), 34.9 (CH_2 (α)), 178.4 (COOR)
4	0.9 (t, CH_3), 1.1 (d, CH_3),1.6 (m, CH_2 (β)), 2.4 (m, CH (α))	-
5	0.8 (t, CH_3), 1.3 (m, CH_2 (γ)),1.6 (m, CH_2 (β)), 2.4 (m, CH_2 (α))	13.1 (CH_3), 21.7 (CH_2 (γ)),, 26.6 (CH_2 (β)), 33.7 (CH_2 (α)), 179.56 (COOR)
7	1.7 (m, $(CH_2)_2$), 2.4 (t, CH_2 (α)), 3.6 (t, CH_2Cl)	20.8 (CH_2 (β)), 30.7 (CH_2 (α)), 33.5 (CH_2 (γ)), 61.4 (CH_2Cl), 178.87 (COOR).
9	1.65 (m, $(CH_2)_2$), 2.4 (t, CH_2 (α)), 3.6 (t, CH_2Br)	20.8 (CH_2 (β)), 30.8 (CH_2 (α)), 33.6 (CH_2 (γ)), 61.4 (CH_2Br), 174.8 (COOR)
10	1.2 (m, $(CH_3)_2$), 3.65 (s, CH_2Cl)	-
11	0.9 (t, CH_3), 1.3 (m, $(CH_2)_2$), 1.6 (m, CH_2 (β)), 2.4 (t, CH_2 (α))	-
13	1.8 (s, CH_3), 5.8 (br, CH), 6.3 (br s, CH=CH), 7.3 (br s, CH (α))	-
14	1.3 (m, CH_2 (γ)), 1.7 (m, $(CH_2)_2$ (β,δ)), 2.4 (t, CH_2 (α)), 3 (t, $\underline{CH_2}NH_2$)	24.2 (CH_2 (γ)), 25.5 (CH_2 (β)), 26.7 (CH_2 (δ)), 34 (CH_2 (α)), 40 ($\underline{C}H_2NH_2$), 178.9 (COOR)
16	0.1 (t, CH_3), 1.4 (br m, $(CH_2)_3$),1.7 (q, CH_2 (β)), 2.5 (t, CH_2 (α))	13.3 (CH_3), 22.2 (CH_2 (ε)), 24.5 (CH_2 (β)), 28.7 (CH_2 (γ)), 31.3 (CH_2 (δ)), 33.7 (CH_2 (α)), 179 (COOR)
18	0.8 (t, CH_3), 1.3 (br m, $(CH_2)_4$), 1.6 (br m, CH_2 (β)), 2.4 (t, CH_2 (α))	-
20	0.9 (t, CH_3), 1.3 (br m, $(CH_2)_5$), 1.6 (m, CH_2 (β)), 2.3 (t, CH_2 (α))	13.7 (CH_3), 22.6 (CH_2), 24.6 (CH_2 (β)), 29.2 ((CH_2)$_3$), 31.9 (CH_2), 33.8 (CH_2 (α)), 179.4 (COOR)
22	0.9 (t, CH_3), 1.3 (br m, $(CH_2)_6$), 1.7 (br m, CH_2 (β)), 2.3 (t, CH_2 (α))	-

The δ (ppm) data of the OAC derivatives refers to the substituent groups.

O-chitosan derivatives were already synthesized and reported elsewhere [40,41] but the present study has a major advantage, since the acid groups are specifically introduced onto the OH groups of chitosan without an additional protection step of the amino group. In addition, the reaction methods to prepare *O*-chitosan derivatives, proposed in the previous studies [23,25] were rather complex, and resulted mostly in a mixture of *N,O*-chitosan derivatives.

The successful preparation of *N,O*-(acyl) chitosan in $MeSO_3H$ as a solvent has also been described [25,42-44]. However, the problem with this method is the decreasing molecular weight of chitosan by the depolymerization process during the reaction method. A noteworthy point is that both moderate substitution of *N,O*-(acyl) groups and moderate molecular weight are important factors in obtaining high biologically active chitosan derivatives.

An efficient procedure to prepare water-soluble *O*-(succinyl) chitosan and *O,O*-(didecanoyl) chitosan was established using a three-step reaction. A phthaloyl group

was firstly chosen as protecting group for the amino group of chitosan, and *O*-succinylation was then completed. Finally, the protective group was removed using hydrazine hydrate [23,45,46]. However, this method needs several steps for the protection and deprotection of the *N*-phthaloyl groups.
In contrast to the above synthetic methods that need several steps to prepare *O*-chitosan derivatives, the method described here introduces a facile synthesis of *O*-chitosan derivatives without protection of the NH_2 group.

Fungicidal activity of OAC derivatives against B. cinerea and P. grisea

The fungicidal activity of OAC derivatives against *B. cinerea* are shown in Table 3 as EC_{50} values with 95% CL. It can be seen that this group of chitosan derivatives shows low to moderate fungicidal activity against the tested fungus. This result can be attributing to the low DS value obtained. *O*-(decanoyl) chitosan (23) at a mol ratio 1:2 (chitosan to decanoic acid) is the most active one in this group (EC_{50} = 1.02 $g.l^{-1}$) (Figure 1) and most of these derivatives represses spore formation at the tested concentrations. Followed in the descending order by compounds 1, 5 and 10 (EC_{50} = 1.08, 1.08 and 1.19 $g.l^{-1}$, respectively). The activity is further decreased when a double bond is introduced in the side chain (see compound 13 vs. 11 and 12).
In addition, Table 3 indicates that the OAC derivatives exhibit an inhibitory effect at a preliminary screening concentration (5 $g.l^{-1}$) against *P. grisea*. The data show that compounds of *O*-(hexanoyl) chitosans (11 and 12), *O*-(octanoyl) chitosans (18 and 19) and *O*-(nonoyl) chitosan (21) are found to inhibit the mycelial growth *in vitro* by 100% at 5 $g.l^{-1}$. However, compounds 11 (Figure 2) and 21 display the highest activity with EC_{50} 1.11 and 1.30 $g.l^{-1}$, respectively. Also, as previously mentioned with *B. cinerea*, compound 11 represses spore formation up to 0.5 $g.l^{-1}$ (Figure 2). As previously discussed with *B. cinerea*, the data also show that the activity is further decreased when a double bond is introduced in the side chain (see compound 13 vs. 11 and 12).
Although chitosan has been studied in several areas especially for fungicidal and bactericidal activity against several fungi and bacteria, information on the fungitoxicity of chitosan derivatives is limited in the literature. Chitosan is already known to interfere with the growth of several phytopathogenic fungi including *B. cinerea* [47-49], but the mechanism by which it affects the growth of the pathogen is still unclear. However, it is believed that chitosan interferes with the negatively charged residues of macromolecules exposed on the fungal cell surface, and thereby changes the permeability of the plasma membrane [50].
Our data also indicated that some compounds inhibited both conidial formation and mycelial growth of *B. cinerea* and *P. grisea* as shown in Figures 1 and 2. This result is of interest if we consider that some commercial antifungal agents act only on spore germination or mycelial growth. It is still not known how these derivatives inhibited conidial germination of *B. cinerea* and *P.grisea*, and blocked the mycelial growth. On the other hand, many fungicides have little or no effect on spore germination but strongly inhibit mycelial growth. Consequently, comparison of the potency of chitosan derivatives as an inhibitor of spore formation with its activity in a mycelial growth assay can provide preliminary information on its mode of action [51].
In general, chitosan inhibits spore germination and radial growth of *B. cinerea* [36], although no complete inhibition even at a concentration of 6 $g.l^{-1}$ was found, indicating that chitosan is more fungistatic rather than fungicidal. This finding is in

agreement with our results, stating that the EC_{50} of chitosan against *B. cinerea* is 5.31 $g.l^{-1}$. In our study, chitosan showed low fungicidal activity against *P. grisea* compared to *B. cinerea.* This finding is in agreement with Liu *et al.,* [28] reporting a MIC of 0.5% of chitosan against *P. grisea* (Ascomycetes). This phenomenon can be explained because this fungus contains chitin and chitosan as major components of its cell wall as previously discussed in the literature [52-54].

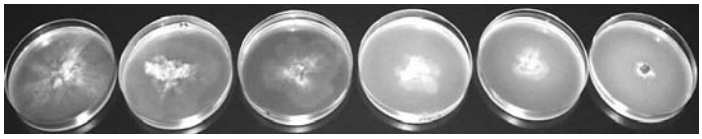


Figure 1. Effect of *O*-(decanoyl) chitosan (23) on the hyphal growth of *B. cinerea* from right to left 4.0, 2.0, 1.0, 0.75 and 0.5 $g.l^{-1}$ and the control.

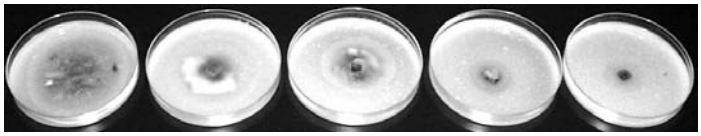


Figure 2. Effect of *O*-(hexanoyl) chitosan (11) on the hyphal growth of P. grisea from right to left 5.0, 2.0, 1.0, 0.5 and 0.1 g.l-1.

Table 3. Fungicidal activity of OAC derivatives against *B. cinerea* and *P. grisea*.

Entry	EC_{50} (95% CL) ($g.l^{-1}$) in *B. cinerea*	Inhibition (%) at 5 $g.l^{-1}$ against *P. grisea*
Chitosan	5.31 (4.07-7.01)	5.13
1	1.08 (0.58-1.93)	5.00
2	1.93 (1.54-2.34)	3.90
3	4.05 (3.51-5.02)	12.83
4	> 6	22.00
5	1.08 (0.70-1.63)	1.00
6	3.22 (2.42-5.42)	14.50
7	> 6	15.10
8	4.17 (2.82-12.6)	15.70
9	> 6	9.64
10	1.19 (1.02-1.39)	15.70
11	3.62 (3.31-4.09)	100 (1.11, 0.79-1.77)*
12	5.52 (4.15-8.79)	100 (5.32, 2.89-18.6)*
13	> 6	8.00
14	> 6	18.40
15	> 6	18.70
16	1.86 (1.49-2.24)	12.10
17	1.37 (1.31-1.41)	26.24
18	4.77 (3.67-8.50)	100 (8.88, 4.44-97.5)*
19	3.81 (3.29-5.16)	100 (2.82, 1.24-6.46)*
20	> 6	13.50
21	1.81 (1.21-2.18)	100 (1.30, 1.10-1.50)*
22	3.20 (2.75-3.95)	31.63
23	1.02 (0.91-1.14)	20.90

* EC_{50} with 95% confidence limits.

Insecticidal activity of OAC derivatives against S. littoralis

The insecticidal and growth inhibitory effects of OAC derivatives against third-instar larvae of *S. littoralis* are given in Table 4. The results show that *O*-(butyroyl) chitosan (2), *O*-(2-methylbutyroyl) chitosan (4), and *O*-(heptanoyl) chitosan (16) produce significant mortalities of > 40% and compound 16 exhibits 57% larval mortality showing the highest insecticidal activity. The results also indicate that most of the synthesized compounds are more active than chitosan but the activity is still low.

As shown in Table 4, these derivatives show low mortality against larvae of *S. littoralis* however all of the OAC derivatives inhibited larval growth stage (> 31% inhibition growth) after 5 days of feeding on treated diet with 5 $g.kg^{-1}$ of each product. High growth inhibition is found after the 1st and the 4th day of treatment for all derivatives and the data also indicate that chitosan itself had a negligible growth inhibitory activity against *S. littoralis*. Moreover, these derivatives show a reduction in larval length and *O*-(2-methylbutyroyl) chitosan (3), *O*-(2-bromo-*iso*-butyroyl) chitosan (9), *O*-(heptanoyl) chitosan (17), and *O*-(decanoyl) chitosan (23) are the most effective compounds on the larval length compared to the control (Figure 3). The food

Table 4. Insecticidal and growth inhibition activity (%) of chitosan and OAC derivatives at 5 $g.kg^{-1}$ against third-instar larvae of *S. littoralis* by feeding on artificial diet.

Entry	Growth inhibition (%) through 5 days of feeding ± SE					% of length ± SE[a]	Mortality (%) ± SE[b]
	1	2	3	4	5		
Control	0	0	0	0	0	100	0
Chitosan	17±0.01	5±0.05	25±0.03	6±0.09	7±0.21	89±0.93	10±5.8 ^{ef}
1	75±0.01	68±0.01	61±0.06	67±0.01	39±0.09	82±0.93	33±6.7 ^{bcde}
2	87±0.01	66±0.02	69±0.05	73±0.07	48±0.06	65±1.14	50±5.8 ^{ab}
3	78±0.01	66±0.02	72±0.01	76±0.03	60±0.11	60±0.93	20±0.0 ^{cdef}
4	21±0.04	69±0.01	51±0.09	64±0.15	47±0.12	71±0.75	47±6.7 ^{ab}
5	18±0.02	49±0.04	68±0.06	70±0.04	48±0.04	66±1.02	40±5.8 ^{abc}
6	56±0.01	55±0.03	72±0.08	73±0.09	56±0.23	62±1.02	33±3.3 ^{bcde}
7	62±0.01	49±0.02	64±0.05	73±0.05	54±0.06	78±0.93	7±6.7 ^{f}
8	69±0.02	63±0.02	72±0.02	75±0.01	59±0.05	70±1.02	7±3.3 ^{f}
9	60±0.01	62±0.04	65±0.11	64±0.16	57±0.18	60±0.81	7±3.3 ^{f}
10	51±0.02	67±0.04	68±0.10	66±0.09	49±0.21	78±0.81	27±3.3 ^{bcdef}
11	65±0.01	54±0.02	72±0.06	73±0.06	45±0.08	66±1.24	33±3.3 ^{bcde}
12	60±0.01	61±0.01	70±0.02	75±0.05	55±0.09	72±0.97	16±3.3 ^{cdef}
13	68±0.02	43±0.06	73±0.07	61±0.10	57±0.12	65±1.05	13±3.3 ^{def}
14	69±0.02	57±0.01	72±0.06	67±0.14	60±0.03	67±1.47	20±5.8 ^{cdef}
15	85±0.01	60±0.05	68±0.02	73±0.05	62±0.04	73±1.02	37±8.8 ^{abcd}
16	58±0.02	53±0.02	72±0.07	70±0.11	31±0.15	67±1.72	57±3.3 ^{a}
17	74±0.01	47±0.02	67±0.07	74±0.09	52±0.22	60±1.12	30±5.8 ^{bcdef}
18	62±0.01	49±0.01	67±0.10	67±0.17	51±0.11	65±1.24	13±3.3 ^{def}
19	67±0.01	59±0.01	67±0.02	72±0.01	55±0.07	78±0.87	13±3.3 ^{def}
20	45±0.02	41±0.02	76±0.08	75±0.17	62±0.04	70±1.16	13±3.3 ^{def}
21	71±0.01	61±0.01	74±0.01	76±0.05	62±0.10	64±0.68	27±6.7 ^{bcdef}
22	61±0.02	53±0.01	72±0.06	74±0.18	59±0.07	73±0.71	16±6.7 ^{cdef}
23	66±0.02	59±0.02	73±0.01	78±0.06	64±0.18	59±0.58	27±3.3 ^{bcdef}

[a] Percentage with respect to control at the 5th day and data are expressed as mean percentages ± SEM based on 3 replicates per tested compound, n=30.

[b] Mortality (%) ± SE at the 7th day of feeding and the values followed by the same letter within a column are not significantly different in a Student-Newman-Keuls (SNK) test, $LSD_{0.05} = 14.22$.

intake was drastically reduced into larvae showing considerable growth retardation. The larvae fed on treated diet showed stunted growth (Figure 3). Typically in intoxicated larvae, the normal ecdysis process was affected showing symptoms of inhibition of feeding and weight gain. These larvae were small compared to the control. As noticed from the literature, the mechanisms could include repellency, disruption of feeding physiology, or chronic toxicity possibly related to insecticidal action [23].

Recently, a different series of *N*-alkyl chitosan and *N*-benzyl chitosan (NBC) derivatives were synthesized and evaluated for their activity against *S. littoralis* [28]. NAC derivatives which had different type of substitutions and mol ratio's were poor in activity, however, good activity was found with NBC derivatives, especially with *N*-(2-chloro-6-fluorobenzyl)chitosan (LC_{50} = 0.32 $g.kg^{-1}$) in artificial diet.

The activity of chitosan and oligo-chitosan was studied on several plant insects [34]. The insecticidal activity of chitosan against *Plutella xylostella* was higher than against *S. exigua*. In addition, chitosan was toxic against *H. armigera* with 38.4 and 40% mortality after 24 and 72h, respectively. However, the mortality was 72% on *P. xylostella*. The mortality of six types of aphids was generally 70-80% against *R. padi*, *M. dirhodum* and *A. gossypii,* while *S. avenae* and *M. persicae* showed a lower susceptibility for chitosan [34].

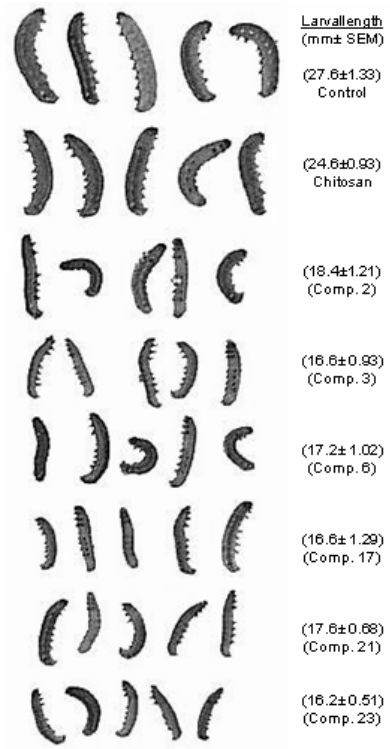


Figure 3. A photograph of larvae of *S. littoralis* at the 5th day reared on artificial diet without treatment (Control) showing normal growth and on a diet containing 5 $g.kg^{-1}$ of chitosan (also showing normal growth as control), 2, 3, 6, 17, 21 and 23 showing stunted growth followed by incomplete shedding of the old cuticle.

Conclusions

Esterification of chitosan could be accomplished successfully in a one-pot reaction. The established procedure enables a facile preparation of *O*-(acyl) chitosan derivatives. The products were obtained with a weak to good DS and a high DS value was obtained with *O*-(butyroyl) chitosan (DS 0.28) at a molar ratio of (1:5) chitosan to butyric acid. All of the synthesized derivatives were soluble in an aqueous diluted triflouroacetic acid (1%). The fungicidal activity was tested against the grey mould *Botrytis cinerea* (Leotiales: Sclerotiniaceae) and the rice leaf blast pathogen *Pyricularia grisea* (Teleomorph: Magnaportha grisea). Most of the OAC derivatives were more active against the two plant pathogens than the native chitosan and the effect was concentration-dependent. The OAC derivatives showed that the insecticidal activity against the cotton leafworm *S. littoralis* is limited but exhibited a strong growth inhibitory action. *O*-(decanoyl) chitosan showed the stronger effect on the larval length and growth compared to chitosan after 5 days of feeding on a treated artificial diet.

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References

1. Francis SJK, Matthew HWT (2000) Biomat 21: 2589
2. Ravi Kumar NVM (2000) React Funct Polym 46: 1
3. Sigh DK, Ray AR (2000) Macromol Chem Phys C40: 69
4. Li Q, Dum ET, Grandmaison EW, Goodman MFA (1992) J Bioact Compat Polym 7: 370
5. Chandy T, Sharma CP (1990) Biomat Art Cells Art Org 18: 1
6. Marguerite R, Pham LD, Claude G (1992) Int J Biol Macromol 14: 122
7. Muzzarelli RAA, Tanfani F (1985) Carbohydr Polym 5: 297
8. Kas HSJ (1997) Microencapsulation 14: 689
9. Kubota B, Kikuchi Y In Polysaccharides: Structural Diversity and Functional Versatility; Dumitriu S, Ed Marcel Dekker New York (1998) p 595
10. Riccardo A, Muzzarelli A (1988) Carbohydr Polym 8: 1
11. Lillo LE, Matsuhiro B (1997) Carbohydr Polym 34: 397
12. Sashiwa H, Kawasaki N, Nakayama A, Muraki E, Yajima H, Yamamori N, Ichinose Y, Sunamoto J, Aiba S (2003) Carbohydr Res 338: 557
13. Skorik Y, Gomes CAR, Vasconcelos TSD, Yatluk YG (2003) Carbohydr Res 338: 271
14. Holme KR, Perlin AS (1997) Carbohydr Res 302: 173
15. Focher B, Massoli A, Torri G, Gervasini A, Morazzoni F (1986) Macromol Chem 187: 2609
16. Grant S, Blair H, Mckay G (1988) Polym Commun 29: 342
17. Heras A, Rodriguez NM, Ramos VM, Agullo E (2001) Carbohydr Polym 44: 1
18. Ramos VM, Rodriguez NM, Diaz MF, Rodriguez MS, Heras A, Agullo E (2003) Carbohydr Polym 52: 39
19. Rabea EI, Badawy MEI, Rogge TM, Stevens CV, Smagghe G, Höfte M, Steurbaut W In Proceedings of the 9th International Chitin-Chitosan Conference, Montereal, Québec, Canada 2003, p103
20. Wenming X, Peixin X, Qin L (2002) Carbohydr Polym 50: 35
21. Zhishen J, Dongfen S, Weiliang X (2001) Carbohydr Res 333: 1
22. Song Y, Onishi H, Nagai T (1992) Chem Pharm Bull 40: 2822
23. Zhang C, Qineng P, Hongjuan Z, Jian S (2003) Eur Polym J 39: 1629
24. Rabea EI, Badawy MEI, Stevens CV, Smagghe G, Steurbaut W (2003) Biomacromol 4: 1457

25. Badawy MEI, Rabea EI, Rogge TM, Stevens CV, Smagghe G, Steurbaut W, Höfte M (2004) Biomacromol 5: 589
26. Rabea EI, Badawy MEI, Rogge TM, Stevens CV, Smagghe G, Höfte M, Steurbaut W In Proceedings of the 9th International Chitin-Chitosan Conference, Montereal, Québec, Canada 2003, p104
27. Rabea EI, Badawy MEI, Rogge TM, Stevens CV, Smagghe G, Hofte M, Steurbaut W (2003) Comm Agric Appl Bio Sci (Ghent University) 68: 135
28. Rabea EI, Badawy MEI, Rogge TM, Stevens CV, Smagghe G, Höfte M, Steurbaut W (2004) Advances in Chitin Sci 7: 231
29. Liu XF, Guan YL, Yang DZ, Li Z, Yao K,D (2001) J Appl Polym Sci 79: 1324
30. Cabrera G, Casals P, Cardenas G, Taboada E, Neira P (2001) In Proc 10th IUPAC Int Congress Chem Crop Protec 1: 227
31. Cardenas G, Paredes JC, Cabrera G, Casals PJ (2002) Appl Polym Sci 86: 2742
32. Casals P, Cardenas G, Galvez G, Villar A, Cabrera G (2002) In Proc 10th IUPAC Int Congress Chem Crop Protec 1: 228
33. Placencia J, Rudolph A, Cabrera G, Cardenas G, Parra C (2003) Bull Environ Contam Toxicol 70: 53
34. Zhang MI, Tan T, Yuan H, Rui CJ (2003) Bioactive Compatible Polym 18: 391
35. Stossel P, Leuba JL (1984) J Phytopathol 111: 82
36. El Ghaouth EA, Arul J, Grenier J, Asselin A (1992) Phytopathol 82: 398
37. Finney DJ Probit analysis. 3rd Ed. Cambridge University Press Cambridge (1971) p 318
38. Smagghe G, Carton B, Decombel L, Tirry L (2001) Arch Insect Biochem Physiol 46:127
39. Smagghe G, Decombel L, Carton B, Voigt B, Adam G, Tirry L (2002) Insect Biochem Mol Biol 32: 199
40. Kim JH, Lee YM (1993) Polym 34: 1952
41. Jin CW, Kim CH, Choi KS (1995) J Korean Ind Eng Chem 6: 482
42. Sashiwa H, Norioki K, Atsuyoshi N, Einosuke M, Noboru Y, Hong Z, Hiroshi N, Yoshihiko O, Hiroyuki S, Yoshihiro S, Sei-ichi A (2002) Biomacromol 3: 1120
43. Sashiwa H, Norioki K, Atsuyoshi N, Einosuke M, Noboru Y, Sei-ichi A (2002) Biomacromol 3: 1126
44. Seo T, Ikeda Y, Torada K, Nakata Y, Shimomura Y (2001) Chitin Chitosan Res 7: 212
45. Inoue K, Yoshizawa K, Othto K, Nakagawa H (2001) Chem Lett 30: 698
46. Nishimura S, Kohgo O, Kurita K, Vittavatvong C, Kuwahara K (1990) Chem Lett 19: 243
47. Du J, Gemma H, Iwahori S (1997) J Jpn Soc Hortic Sci 66: 15
48. El Ghaouth A, Arul J, Wilson C, Asselin A, Benhamou N (1994) Mol Plant Pathol 44: 417
49. Oh SK, Cho D, Yu SH (1998) Korean J Plant Pathol 14: 278
50. Benhamou N (1996) Trends Plant Sci 1: 233
51. Debieu D, Bach J, Hugon M, Malosse C, Leroux P (2001) Pest Manag Sci 57: 1067
52. Allan CR, Hadwiger LA (1979) Exp Mycol 3: 285
53. Hon DNS In: Polysaccharides in Medicinal Application; Dumitri S Ed Marcel Dekker: New York (1996) p 631
54. Yokoi H, Aratake T, Nishio S, Hirose J, Hayashi S, Takasaki Y (1998) J Ferment Bioeng 85: 246