Insecticidal and Fungicidal Activities of Chitosan and Oligo-chitosan

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ABSTRACT: The activity of chitosan and oligo-chitosan on several plant insects and pathogens were studied. Chitosan was active against lepidopterous and homopterous insects; the mortality was 80%. The insecticidal activity of chitosan to *Plutella xylostella* L. was higher than that to *Spodoptera exigua* Hübner. The mortality of six types of aphids was generally 60–80%, and the highest was 99.7%. Some fungicidal activity by oligo-chitosan was observed on 12 plant pathogens. The inhibition effect of oligo-chitosan to plant pathogens was improved with increasing oligomer concentration. The highest inhibition rate (80%) was against *Phomopsis asparagi* (sacc.a) Bubak (95%) and the inhibition rate against *Fusarium oxysporum* (Schl.) f. sp. *Cucumernum owen*, *Rhizoctonia solani* Kuhn and *Fusarium oxysporum* Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans.

KEY WORDS: chitosan, oligo-chitosan, insecticidal activity, fungicidal activity, pathogens, oligomer.

INTRODUCTION

Chitosan, the product of deacylated chitin, is one of the most abundant, renewable materials in the world. Due to its safety and

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biodegradability, and environmental compatibility, the chitosan has been found to have many applications in biology, cosmetics, food, wastewater treatment, heavy metal recovery, photographic chemistry, textile weave, printing, dyeing, and paper making [1,2]. Chitosan also has wide applications in medicine, such as, drug delivery, wound healing and dressing, anticholestestermic agents and antibacterial and antivirus [2,3]. Chitosan has antibacterial activity and it has been used in food and fruit preservation [4]. Einosuke found that chitosan oligosaccharides, with a degree of polymerization (DP) of 6–8, have antitumor activity [5].

Recently, the application of chitosan and chitin agriculture is becoming a major focus of research. Since most chemical pesticides are highly toxic to humans and animals and not very biodegradable, they often cause water and soil pollution. A sustainable healthy environment needs ''green'' pesticides. Research has shown that chitosan and its oligomers can be used as the plant growth regulators, fungicides, and seed coating agents by the induction of phytoalexins. Benhamou et al. studied the resistance of tomato plants to the root rot pathogen *Fusarium oxysporum* and found that at concentrations ranging from 0.5 to 2 mg/mL of chitosan the protection to fungal attack was enhanced [6]. Ghaouth reported that chitosan has antifungal activity on several postharvest pathogens [7]. Akiyama found that chitosan oligomers (DP of 7–8) are potential elicitors for *Pisatin* induction in Pea epicotyls [8]. Although several researchers have reported antifungal activity of chitosan, few have studied the anti-insect pest activity of chitosan.

The present study is focused on killing insect pests and resisting fungi using the chitosan solutions whose main ingredient is either macromolecule chitosan or oligo-chitosan.

EXPERIMENTAL

Materials, Insects, and Plant Pathogens

Chitosan and oligo-chitosan were made in our laboratory with 80% deacylated chitosan. The concentration of the stock chitosan solution was 60 mg/mL in a 1% acetic acid solvent. The molecular weight of chitosan was 300,000. The chitosan oligomers were prepared in our laboratory and the DP of the oligo-chitosan, segment distribution from monosaccharide to the oligomers, was 20 (weight percent of DP 6–8 is 12.25%). The oligo-chitosan was dissolved in water and the molecular weight distribution determined according to He [9].

Insects Tested

Helicoverpa armigera, *Plutella xylostella*, *Rhopalosiphum padi*, *Sitobion avenae*, *Metopolophium dirhodum, Myzus persicae, Hyalopterus prun,* and *Aphis gossypii* were supplied by the Institute of Plant Protection, Chinese Academy of Agriculture Sciences.

Plant pathogens tested were: *Rhizoctonia solani* Kuhn, *Fusarium oxysporum* (Schl.) f. sp. *Cucumernum owen*, *Cladosporium cucumerinum* Ell. Et Arthur, *Botrytis cinerea* Pers., *Colletotrichum orbiculare* (Berk. & Mont.) Arx, *Phomopsis asparagi* (sacc.a) Bubak, *Alterneria Kikuchiama Tanaka, Penicillum italicum Wehmer, Rhizoctonia solani* Kuhn., *Fusarium oxysporum* Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans., *Verticllium ctahliae* Kleb., *Rhizoctonia sclani* Kuhn., *Botryosphaeria berengeriana de Not*. f. Sp. *Piricola* (*Nose*) *Koganezaea et Sakuma, Sclerotinia sclerotiorum* (Lib.) de Bary, *Venturia nashicola Tanaka et Yamamoto*, *Gibberella zeae* (Schw.) *Petch and Phytophthora infestabs* (Mont.) de Bary were supplied by the Institute of Plant Protection, Chinese Academy of Agriculture Sciences.

Bioassay of Anti-insect Activity

(a) Insecticidal Activity to Helicoverpa armigera The chitosan solution was diluted to 3 g/L, then poured into a hand-held sprayer. *Helicoverpa armigera* larva was placed on a piece of cole leaf (insect number 60). The leaf was sprayed with the diluted solution and dried in an experiment box with ten holes under natural environmental conditions. The mortality was tested after one day and three days, respectively.

(b) Insecticidal Activity to Plutella xylostella The stock chitosan solution was diluted to 3 (and 1.2) g/L, then poured into hand-held sprayer. *Plutella xylostella* larva were set on a cole leaf (insect number 60). The leaf was sprayed with the diluted solution and dried in an experiment box with ten holes under natural environmental conditions and moved into a culture container and covering them with fresh plastic membrane. The mortality was tested after 48 h.

(c) Insecticidal Activity to Aphids The stock chitosan solution was diluted to 600, 800, 1200, 3000, and 6000 mg/L and used as prescribed. Cole leaves were inoculated with different aphids including; *Rhopalosiphum padi* (insect number 60), *Sitobion avenae* (insect number 96), *Metopolophium dirhodum* (insect number 125), *Myzus persicae* (insect number 391), *Hyalopterus prun* (Göffroy) (insect

number 391), *Aphis gossypii* (Glover) (insect number 228) then dipped in the dilute chitosan, or sprayed with the diluted chitosan solutions. The leaves were dried in a natural environment, moved into culture container and covered with a fresh plastic membrane. After 24 and 48 h, respectively, the mortality was tested.

Evaluation of Mortality

If the larva does not move when touched, it was considered dead. The mortality (%) was the percent of dead larvae to the total larvae tested. Adjusted mortality rate P was calculated based on the following formula [10]:

$$P = \frac{P_1 - P_0}{1 - P_0} \times 100\%$$

P_1 is the mortality rate of chitosan solution treatment, P_0 is the mortality rate of control.

Bioassay of Fungicidal Activities *In Vitro*

(a) Fungi Culture Petri dishes (8 cm diameter) were sterilized in an autoclave; then a culture medium (potato starch 200 g, glucose 20 g, agar 18–20 g in 1000 mL of water) [10] was poured into the dishes without fungi. The fungi to be tested were added, respectively, to the dishes and cultured at $24^\circ C \pm 0.5^\circ C$ for seven days. When the plates were full of mycelia, culture agro cakes were cut from the edge of the above cakes with a hole-making tool (6 cm).

(b) Preparation of Culture Medium Containing Chitosan Five different chitosan stock concentrations were prepared under asepsis conditions. The chitosan solutions were added, respectively, to the fungi culture medium prepared above heated to $40^\circ C$; this mixture was added to a series of sterilized 8 cm petri dishes. Each chitosan concentration solution was replicated in four plates and the average result was taken. The antifungal activity was between 10 and 90%, respectively.

(c) Inoculation and Culture Culture medium (5 mL) containing chitosan was added to the Petri dishes inoculated with fungi [(b) above] under sterilized environment. These were cultured at $24^\circ C \pm 0.5^\circ C$. After the mycelia was completely grown, the diameter of each mycelia was measured and the antifungal activity was calculated.

(d) Calculation of Antifungi Activity Each sample was measured two times and the averaged value was taken. The mycelia diameter and anti-fungi activity was calculated using the formula:

$$I = \frac{\overline{D_1}^2 - \overline{D_0}^2}{\overline{D_1}^2} \times 100\%$$

I is the antifungal activity index (%), $\overline{D_1}$ is the average diameter of control sample with fungi, $\overline{D_0}$ is the average diameter of tested fungi.

From a biology statistic probability table [10], the control percent to control probability were regulated and the concentration logarithm as abscissa and constrain rate as ordinate was determined, and then the concentration for 50% fungi inhibition (EC_{50}) and the concentration for 90% fungi inhibition (EC_{90}) were calculated.

RESULTS AND DISCUSSION

Insecticidal Activity of Chitosan to *Helicoverpa armigera*

Using a 3 g/L chitosan solution sprayed on an infected leaf, the mortality was 38.4% after 24 h; after 72 h the mortality was 40.0% (Table 1). The control leaf without chitosan had no insecticidal activity. The anti-insect activity of chitosan solution was less than that of the chemical pesticide phoxim which exhibited 76.4% mortality after 72 h under similar concentrations. Phoxim is an organic phosphate insecticide with the highest activity commercially available. Although it is widely used, phoxim is toxic to humans and animals. However, the chitosan solution has no apparent toxic effects.

The chitosan oligomers had lower insecticidal activity. The reason for the high insecticidal activity by chitosan and low activity with oligomers is not clear. Perhaps, chitosan forms a film on the surface of insects and the film can block air from the insects and the insects die. Some researcher has suggested that chitosan induces chitosanase

Table 1. Insecticidal activity of chitosan on Helicoverpa armigera.

	Adjusted Mortality Rate (P)	
Insecticide Used	24 h (%)	72 h (%)
Chitosan solution	38.4	40.0
CK	0	3.3
Phoxim	73.3	76.4

activity in the insect's body causing insecticidal activity [11]. The oligomers may not have this function.

Insecticidal Activity of Chitosan to *Plutella xylostella*

The chitosan solution has a better insecticidal activity to *Plutella xylostella* (Table 2), and the 3 g/L solution had much higher insecticidal activity than the 1200 mg/L solution. The insecticidal activity of 3 g/L solution reached 72%, which is comparable with commercial chemical pesticides.

Insecticidal Activity of Chitosan to Aphids

From our experimental data, chitosan has different insecticidal activity to various aphids (Figure 1). For example, chitosan has a very high insecticidal activity for *Hyalopterus prun* (Goffroy) on flowers; the emendation mortality was always between 93 and 99%. If we increased the concentration of chitosan solution from 600 to 6000 mg/L, for the aphid on the cole the emendation mortality was only 20.2% for the

Table 2. Insecticidal activity of chitosan on Plutella xylostella.

	Chitosan Solution			Phoxim
Test Time (h)	3 g/L (%)	1.2 g/L (%)	CK	0.5 g/L (%)
48	62	40	0	79.2
72	72	44	0	87.5

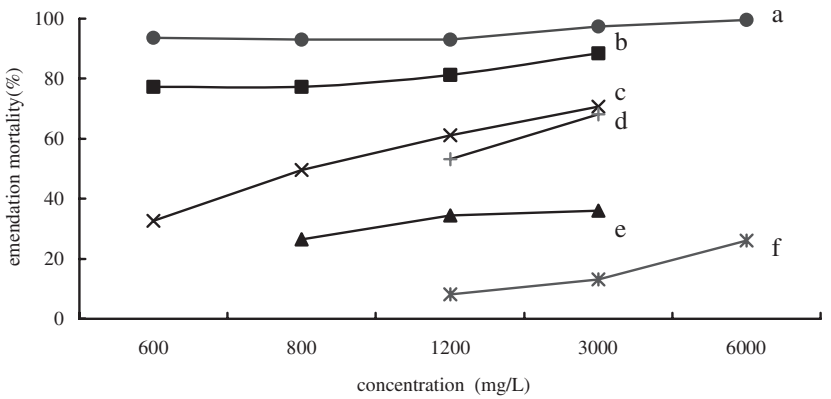

Figure 1. Insecticidal activity of chitosan to aphid. (a: *Rhopalosiphum padi* (L.); b: *Sitobion avenae* (Fabricius); c: *Metopolophium dirhodum* (Walker); d: *Myzus persicae* (Sulzer); e: *Hyalopterus prun* (Göffroy); f: *Aphis gossypii* (Glover)).

aphids on the cole with same concentration. Chitosan had 70–80% insecticidal activity against *Rhopalosiphum padi, Metopolophium dirhodum* (*Walker*) and *Aphis gossypii* (*Glover*), however, only *Sitobion avenae* (*Fabricius*) and *Myzus persicae* (*Sulzer*) chitosan had a low insecticidal activity.

Fungicidal Activity of Chitosan to Plant Fungus

Using the biology statistic probability table [10] and the data in Table 3, inhibition percent was changed into inhibition probability. A line was made for the logarithm concentration and inhibition probability (Figure 2). The correlation coefficients were obtained and then the EC_{50}s of chitosan solution to *Phompsis asparagi* (sacc.a) *Bubak*, *Fusarium oxysporun* Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans. and *Verticllium ctahliae* Kleb was calculated. The results show that chitosan has an EC_{50} of 626.78, 393.16, and 854.19 μg/L, respectively, for the three fungi (Figure 2). Chitosan has the lowest EC_{50}, namely best constrain function, for *Fusarium oxysporun* Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans.

Table 3. Antifungal activity of oligo-chitosan on plant fungus.

	Oligo-chitosan Concentration (mg/L)				
Plant Pathogens	20	30	50	100	150
Rhizoctonia solani Kuhn. %	77.8	49.0	0	0	0
Fusarium oxysporum (Schl.) f. sp. *Cucumernum* owen, %	84.0	75.0	55.6	36.0	0
Cladosporium cucumerinum Ell. Et Arthur, %	40.8	28.4	14.8	0	0
Botrytis cinerea Pers.	55.6	46.2	24.9	0	0
Colletotrichum orbiculare (Berk. & Mont.) Arx %	58.7	49.0	32.5	20.3	0
Phomopsis asparagi (sacc.a) Bubak %	95.1	55.6	39.5	0	0
Alterneria Kikuchiama Tanaka %	75.0	67.9	55.6	30.6	12.9
Penicillum italicum Wehmer %	0	0	0	0	0
Rhizoctonia solani Kuhn. %	89.6	87.4	58.4	49.6	0
Fusarium oxysporum Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans. %	84.0	75.0	55.6	36.0	0
Verticllium ctahliae Kleb. %	67.3	49.0	26.5	0	0
Rhizoctonia sclani Kuhn. %	77.8	49.0	0	0	0
Botryosphaeria berengeriana de Not. f. Sp. Piricola (*Nose*) *Koganezaea et Sakuma*, %	39.0	28.8	23.4	17.9	0
Sclerotinia sclerotiorum (Lib.) de Bary, %	57.5	38.8	24.4	0	0
Venturia nashicola Tanaka et Yamamoto, %	30.6	10.8	0	0	0
Gibberella zeae (Schw.) Petch, %	75.0	68.4	60.9	52.7	0
Phytophthora infestabs (Mont.) de Bary, %	46.2	41.2	19.0	12.9	0

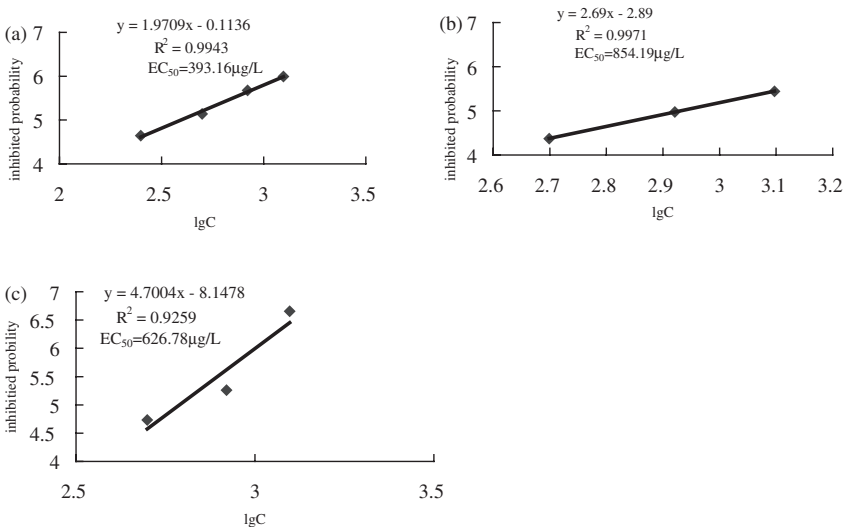

Figure 2. Relationship between inhibition probability and concentration logarithm. (a) *Fusarium oxysporum* Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans.; (b) *Verticllium ctahliae* Kleb; (c) *Phomopsis asparagi* (sacc.a) Bubak.

When the chitosan stock solution (2500 mg/L) was diluted 20, 30, 50, 100, 150, 200 times, it had antifungal function to 16 fungi except to *Penicillum italicum* Wehmer. Moreover it has higher functions at higher concentrations. When the solution was diluted 20 times, it still had 95.1% antifungal activity to *Phomopsis asparagi* (sacc.a) Bubak. To *Fusarium oxysporum* (Schl.) f. sp. *Cucumernum owen, Rhizoctonia sclani* Kuhn and *Fusarium oxysporum* Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans, the solution diluted by 20 times, was over 80% antifungal activity to many of the general virus fungi causing plant diseases.

Benhamou et al. reported that chitosan enhanced the resistance of tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f. sp [6]. The optimal concentration was 2 mg/L. Based on our results, chitosan has a wide antifungal activity for pathogenic fungi. The antifungal activity of chitosan may be based on chitosan-induced resistance which enhances protection to the plant. The chitosan oligomers interact with the walls of invading hyphae and induces extra cellular chitosanase which has antifungal activity. The oligomer activity is shown in Figure 3. When the oligomer was diluted 20–30 times (125–167 mg/L), respectively, as shown in the plates in the top row in Figure 3, the medium looked very clear which means that all the fungi, including *Botrytis cinerea* Pers (A) and *Rhizoctonia sclani* Kuhn (B) were inhibited at least 99%. The medium for the controls (CK) (2 plates in

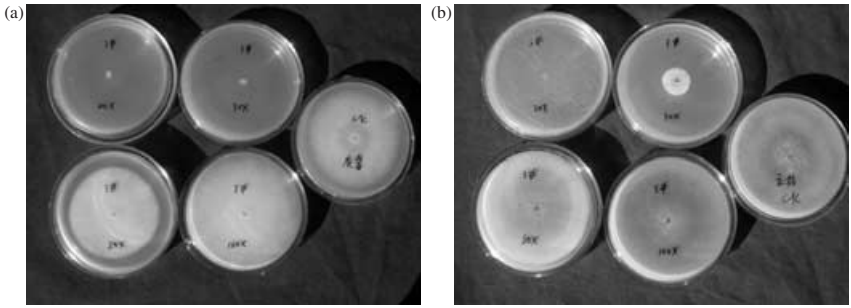

Figure 3. Antifungal activity of oligomer of chitosan: (A) *Botrytis cinerea* Pers and (B) *Rhizoctonia sclani* Kuhn.

the middle row in Figure 3), looked very turbid which indicated fungi grown on these plates. When the oligomer was diluted 50 times or higher, the fungi (partial or all turbid plates in the bottom row in Figure 3) was not effectively inhibited.

CONCLUSIONS

The chitosan has high insecticidal activity for lepidopterous and homopterous insect pest; it has higher activity to *Plutella xylostella* L. than to *Helicoverpa armigera* Hübner and *Spodoptera exigua* Hübner; Chitosan has the ability to kill small pest (aphid), especially *Hyalopterus prun*. Although chitosan has less killing ability than the chemical pesticide phoxim, due to its low toxicity and environment compatibility, it could be used on vegetables and fruits as a substitute for the toxic chemical pesticides now being used.

Oligo-chitosan has good inhibition effects against 16 different fungi, especially *Fusarium oxysporum* Schl. F. Sp. *Uasinfectum* (Atk.) Snyd. & Hans., *Verticllium ctahliae* Kleb., *Rhizoctonia sclani* Kuhn., *Phomopsis asparagi* (sacc.a) Bubak, *Alterneria Kikuchiama Tanaka, Rhizoctonia solani* Kuhn., *Gibberella zeae* (Schw.) Petch, *Colletotrichum orbiculare* (Berk. & Mont.) Arx *and Sclerotinia sclerotiorum* (Lib.) de Bary. Oligo-chitosan has good characteristics as a noncontaminant, biologically, degradable and environment compatible, therefore, chitosan and its oligomers have a potential as a pesticide due to their ''green'' chemical properties.

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