Insecticidal Activity of Chitosans of Different Molecular Weights and Chitosan-metal Complexes against Cotton Leafworm *Spodoptera littoralis* and Oleander Aphid *Aphis nerii*

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Abstract

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As an alternative to synthetic pesticides, chitosan has received much attention as a biopolymer active against some agricultural pests. The, insecticidal activity of chitosans of four molecular weights (2.27×10^5, 3.60×10^5, 5.97×10^5, and 9.47×10^5 g/mol) was investigated against two species of arthropod pests: oleander aphid *Aphis nerii* and cotton leafworm *Spodoptera littoralis*. In addition, the most active chitosan of 2.27×10^5 g/mol was chemically modified with metals of Ag(I), Cu(II), Ni(II), and Hg(II) to give corresponding chitosan-metal complexes. Larval mortality, growth inhibition, and antifeedant activities for third instar larvae of *S. littoralis* were evaluated at 4 g (a.i.) chitosan/kg diet. Chitosan of 2.27×10^5 g/mol and its complexes with Ni and Hg were the most active compounds. The results against *A. nerii* indicated that chitosans of 3.60×10^5 and 5.97×10^5 g/mol showed high activity among the different molecular weights in leaf-dip bioassay after 24 h of treatment with 48 and 49% mortalities, respectively, at 1000 mg/l. All compounds had a systemic effect against *A. nerii*. Chitosans of 2.27×10^5, 3.60×10^5, and 5.97×10^5 g/mol showed the highest efficacy at all concentrations tested; however, chitosan-Cu was significantly the most active among the complexes.

Keywords: arthropod pests; larval mortality; biopolymer

Chitosan is derived by deacetylation of chitin, the second most abundant natural biopolymer. Chitosan is a nontoxic copolymer consisting of β-(1,4)-2-acetamido-2-deoxy-D-glucopyranosyl and β-(1,4)-2-amino-2-deoxy-D-glucopyranosyl units. It is a large family of compounds with different properties depending on its structure (No & Meyers 1997). The degree of *N*-acetylation (DA) and molecular weight are important factors that have an impact on properties such as solubility (Kubota & Equchi 1997) and biological activity (Rabea *et al*. 2003; Gerasimenko *et al*. 2004; Badawy 2008, 2010). Thus, the free amino groups on chitosan molecule are important for many applications.

Recently, chitosan-metal complexes have attracted great interests for their potential use in agriculture, medical industry, and food industry (Nieto *et al*. 1992; Shigemasa & Minami 1996; Wang *et al*. 2004; Mekahlia & Bouzid 2009; Higazy *et al*. 2010). It is well known that both chitosan and metals such as Ag, Cu, Ni, Hg, and Zn have the properties of disinfection and bactericide (Wang *et al*. 2005). After chitosan binds to metal ions through nitrogen, oxygen or a combination, the bindings are likely to leave some potential donor atoms free and these free donor atoms enhance biological activity (Wang *et al*. 2005). However, the influence of the property of metal ions, molecular parameters and environmental factors on

the biological activity of chitosan–metal complexes is currently unclear.

Spodoptera littoralis (Boisduval) is an important polyphagous pest, widely distributed all over the world (Brown & Dewhurst 1975). Larvae of this pest can feed on ~90 economically important plant species belonging to 40 families (Scarpellini 2001). The oleander aphid *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae), sometimes called the milkweed aphid, is a common pest of several ornamental plants in the families Apocynaceae and Asclepiadaceae. The damage caused by aphid colonies is mainly aesthetic due to the large amounts of sticky honeydew produced and the resulting black sooty mould that grows on the honeydew. It is able also to transmit several viruses (Hall & Ehler 1980; Blackman & Eastop 2000).

The use of synthetic pesticides in the control of such pests resulted in potential hazards for mammals and non-target organisms, disturbances of the environment, pest resistance (Prakash & Rao 1987). Therefore, at present there is a need for novel physiologically active compounds as safe alternatives to harmful pesticides in order to overcome resistance and compatible with integrated pest management practices. In addition, such compounds should possess a high selectivity so that they can be used without danger for humans, fish, beneficial organisms and the environment in general. As a consequence, chitosan may serve as a good alternative for broad-spectrum and highly persistent pesticides because it is non-toxic to vertebrates and humans, biodegradable, and may possess insecticidal and microbicidal properties (Rabea *et al*. 2003; Badawy *et al*. 2005).

The present study is to investigate the insecticidal activity of chitosan of different molecular weights and chitosan-metal complexes against cotton leafworm *Spodoptera littoralis* and oleander aphid *Aphis nerii* as an example of sucking pest. The economic importance of this aphid is mostly based on their destructiveness to agricultural plants, especially to ornamental plants, in the families Apocynaceae and Asclepiadaceae. It is commonly found worldwide feeding on oleander, *Nerium oleander*, milkweeds. *Aphis nerii* quickly grow to huge numbers on host plants and cause a decline in the health of the plant as damage is generally restricted to unsightly sooty mould caused by secretion of honeydew (Hall & Ehler 1980).

MATERIAL AND METHODS

Materials. Chitosans of 2.27×10^5, 3.60×10^5, $5.9\ 7 \times 10^5$, and 9.47×10^5 g/mol were purchased from Sigma-Aldrich Co. (St. Louis, USA). Acetone, acetic acid, potassium bromide (KBr), silver nitrate ($AgNO_3$), copper sulphate ($CuSO_4 \cdot 5H_2O$), nickel chloride ($NiCl_2 \cdot 6H_2O$), and mercuric chloride ($HgCl_2$) were purchased from Adwic El-Nasr Pharmaceuticals Chemical Co. (Cairo, Egypt). Soybean-wheat germ insect artificial diet (Manduca Premix-Heliothis Premix) was purchased from Stonefly Ind. (Bryan, Texas).

Preparation of chitosan-metal complexes. Chitosan-metal complexes were prepared according to the method described by Wang *et al*. (2004). Chitosan (10 mmol, 1.61 g calculated as glucosamine unit) was dissolved in 50 ml aqueous acetic acid (1%). The exact known concentration of $AgNO_3$, $CuSO_4 \cdot 5H_2O$, $NiCl_2 \cdot 6H_2O$, and $HgCl_2$ pre-dissolved in distilled water was added to the chitosan solution (corresponding to a molar ratio of 1:1 compared with chitosan residue). After addition of the salt, the pH value was increased to 7.0 by adding 0.1M NH_4OH solution. The mixture was refluxed at 80°C for 3 h with stirring. After cooling to room temperature, the mixture was poured into 200 ml acetone. The resulting precipitate was obtained by filtration. The product was repeatedly washed with acetone and then oven-dried overnight at 60°C producing the chitosan-metal complexes. IR spectra of chitosan and chitosan-metal complexes were recorded using Perkin Elmer's Spectrum RXIFT-IR spectrophotometer.

Test pests. A susceptible strain of the cotton leaf worm *S. littoralis* Boisduval (Lepidoptera: Noctuidae) was used for bioassay. This strain was reared for many years in a laboratory of Economical Entomology Department, Faculty of Agriculture, Alexandria University, Egypt, without exposure to any pesticides. The colony was reared under laboratory conditions on artificial diet under controlled conditions at 25 ± 2°C, 70 ± 5% relative humidity (RH) and a 16 h light photoperiod (Eldefrawi *et al*. 1964). Samples of *A. nerii* Boyer de Fonscolombe (Hemiptera: Aphididae) were collected at the infested oleander plant (*Nerium oleander*, Antonyadis garden, Alexandria Governorate, Egypt, between March and April 2010). The aphid pest was tested directly without further rearing.

Insecticidal activity against *S. littoralis*. In a standardised screening toxicity test, third-instar

larvae of *S. littoralis* were selected from the laboratory colony. Chitosan compounds were dissolved in 37.5 part of 1% (v/v) aqueous acetic acid for 24 h at room temperature and then incorporated with 12.5 part of an artificial diet to give a final concentration of 4 g (a.i.) chitosan/kg diet. Untreated diet was provided to controls. Treated diet was divided and placed in Petri dishes. Three replicates for each treatment and control and 10 larvae were introduced onto each replicate. The experiments were kept in a growth chamber, at 25 ± 2°C, 70 ± 5% RH and a 16 h light photoperiod. After 7 days of continuous feeding, larval mortality was scored; if no movement was observed, larvae were considered as dead. Larval growth inhibition was assayed relative to the control based on larval weight gain through 7 days of feeding. The growth inhibition was calculated from the following equation:

$$\text{Growth inhibition (\%)} = \left(\frac{C_L - T_L}{C_L}\right) \times 100$$

where:

C_L – larval weight gained in the control

T_L – larval weight gained in the treatment

The percentage of feeding inhibition was determined after 7 days by the formula of Abivardi and Benz (1984):

$$\text{Antifeedant (\%)} = \left(\frac{C - T}{C}\right) \times 100$$

where:

C – weight of diet consumed in untreated control

T – weight of diet consumed in treatment

Insecticidal activity against *A. nerii* ***by leaf-dip method***. Chitosan compounds were dissolved in 50 ml of 0.5% (w/v) aqueous acetic acid and diluted to obtain the required concentrations (250, 500, and 1000 mg/l). To measure the activity of the chitosan compounds against *A. nerii*, fully expanded true leaves from an oleander (*N. oleander*) plant were dipped in chitosan solutions for 30 s and air-dried for 30–60 minutes. The treated leaves were placed on filter papers in Petri dishes (three Petri dishes per each concentration). Once treated leaves had dried, 20 wingless (apterous) adult aphids were transferred with a fine brush to each treatment with three leaves per each replicate. Leaves treated with water were used as a control. The treatments were kept in a constant environment room maintained at 26 ± 2°C, 65 ± 5% RH, with a 12:12 light:dark photoperiod (FAO 1979). Mortality was recorded at 24 and 48 h after treatment under a microscope. Aphids unable to move and showing strong effects of the compound were scored as dead. Mortality values were corrected according to Abbott (1925).

Insecticidal activity against *A. nerii* ***by plant systemic method***. To study the systemic effect of chitosan compounds towards *A. nerii*, branches of oleander plant were transferred to conical flasks (250 ml in volume) containing chitosan solutions at the tested concentration levels of 250, 500, and 1000 mg/l. Newly maturated females from the stock culture were settled on the upper side of the plant leaves (30/branch) and caged for 2 days. Three conical flasks (one branch/flask) were used for each treatment and controls as well. The treatments were kept in a constant environment room maintained at 26 ± 2°C, 65 ± 5% RH, with a 12:12 light:dark photoperiod (FAO 1979). The number of dead aphids was counted after 48 h. Mortality values were corrected according to Abbott (1925).

Statistical analysis. The data were statistically analysed separately for each experiment and were subjected to the analysis of variance (ANOVA) using SPSS 12.0 software (Statistical Package for Social Sciences, USA). Differences between treatment means were established by Student-Newman-Keuls (SNK) test according to Snedecor and Cochran (1989) and differences at $P \leq 0.05$ were considered significant.

RESULTS

Characterisation of chitosans and chitosan-metal complexes

Chitosans of four molecular weights of 2.27×10^5 (Ch 1), 3.60×10^5 (Ch 2), 5.97×10^5 (Ch 3), and 9.47×10^5 (Ch 4) g/mol with 89, 85, 81 and 82% degrees of deacetylation, respectively, were used in the present study (Table 1). Complexes

Table 1. Characterisation of different molecular weights chitosan compounds

Compound	DA (%)	DDA (%)	MW (g/mol)
Ch 1	11	89	2.27×10^5
Ch 2	15	85	3.60×10^5
Ch 3	19	81	5.97×10^5
Ch 4	18	82	9.47×10^5

Ch – chitosan; DA – degree of acetylation; DDA – degree of deacetylation; MW – molecular weight

between low molecular weight (Ch 1) and metals of Ag(I), Cu(II), Ni(II) and Hg(II) were prepared and characterised by IR spectrophotometer. The IR spectra of chitosan–metal complexes as shown in Figure 1 exhibit many alterations from those of unmodified chitosan. The first broad and poorly resolved band around 3400–3450 cm^{-1} corresponds to the contribution of O-H stretching (from intra- and intermolecular hydrogen bonds) and N-H stretching (Wang *et al*. 2000; Tang & Hon 2001). A lot of differences in the spectra before and after metal binding were also observed in the environ-

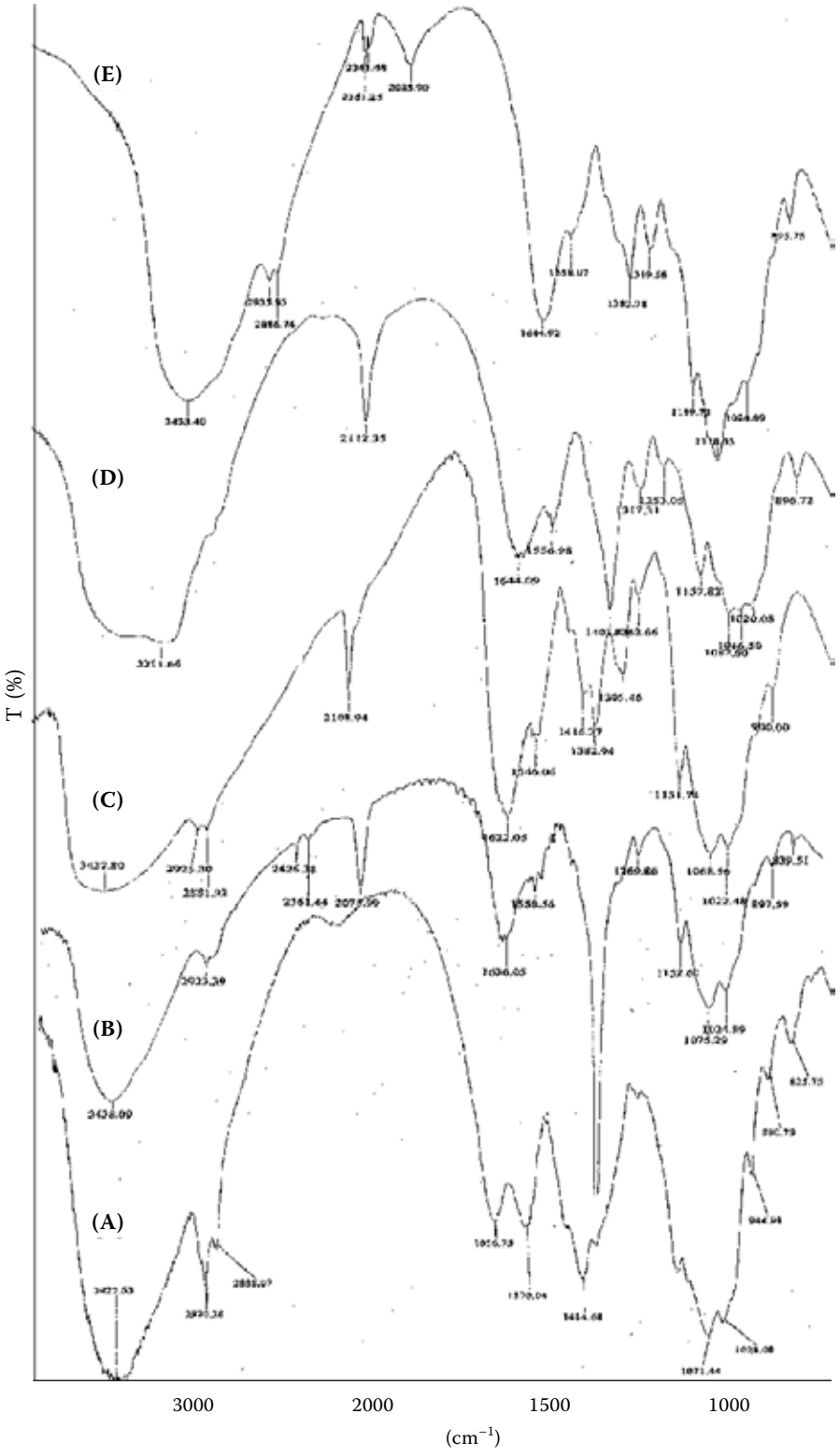

Figure 1. FT-IR spectra of chitosan and chitosan-metal complexes: (**A**) chitosan (Ch 1), (**B**) Ch 1-Ag, (**C**) Ch 1-Hg, (**D**) Ch 1-Ni, and (**E**) Ch 1-Cu

ment of amine and amide groups (amide I band: 1656 cm^{-1} in chitosan; Figure 1A compared to 1622–1644 cm^{-1} in chitosan-metal complexes; Figure 1B-E and amide II band: 1570 cm^{-1} in chitosan compared to 1546–1558 cm^{-1} in chitosan–metal complexes). This group affected or disappeared as shown in spectra in Figures 1B–E, which suggests the amine or the acetamide group at C2 involved in metal binding (Tang & Hon 2001; Mekahlia & Bouzid 2009). Moreover, the intensity of the band at 1414 cm^{-1} in chitosan molecule was substantially affected after metal binding especially in the case of the chitosan-Ag complex (Figure 1B). This peak attributes to bending vibration of -OH, which indicates that -OH takes part in metal binding.

Insecticidal activity against larvae of S. littoralis

Larval mortality, growth inhibition and antifeedant activity of chitosan compounds against third instar larvae of *S. littoralis* are shown in Tables 2 and 3. Low molecular weight chitosan, Ch1 (2.27×10^5 g/mol), exhibited significantly higher larval mortality compared to the other compounds (Table 2). Larval mortality was increased gradually during the feeding of larvae on diet treated with 4 g chitosan/kg diet and 50% mortality was scored with Ch 1 at the end of the experiment (after 7 days). Chitosan-Ni and chitosan-Hg complexes significantly showed high larval mortality (93 and 83%, respectively) at the end of the experiment. In addition, a chitosan-Hg complex proved high toxicity from the second day of feeding. However, chitosan-Ag and chitosan-Cu complexes showed low mortality (20 and 37%, respectively) at the end of the experiment.

The incorporation of chitosan compounds into the diet reduced the mean weight gain of *S. littoralis* larvae compared to the control (Table 3). A comparison between different molecular weights of chitosan, low molecular weight chitosan (Ch 1) was the most active (78% growth inhibition at day 7) among the others. Chitosan-Ni and chitosan-Hg were the most active in larval weight reduction compared to chitosan-Ag and chitosan-Cu. Chitosan-Ni and chitosan-Hg were the most active among chitosan-metal complexes that represent weight reduction or growth inhibition of 97 and 96%, respectively. Chitosan-Cu complex showed moderate larval growth inhibition (54%). However, chitosan-Ag was the least active in larval growth inhibition. Moreover, all of chitosan compounds except the chitosan-Ag complex showed antifeedant activity compared to the control (df = 8, 18; $F = 33$; $P < 0.0001$). Among these compounds,

Table 2. Insecticidal activity of chitosan compounds against third-instar larvae of *S. littoralis* by feeding on 4 g chitosan/kg diet

Treatment	Mortality (%) ± SE through 7 days of feeding				
	1	2	3	4	7
Control	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$0.0^{c} \pm 0.0$	$6.7^{e} \pm 3.3$
Ch 1	$0.0^{b} \pm 0.0$	$23.3^{b} \pm 8.8$	$26.7^{b} \pm 12.0$	$36.7^{b} \pm 12.0$	$50.0^{b} \pm 5.8$
Ch 2	$0.0^{b} \pm 0.0$	$10.0^{b} \pm 0.0$	$20.0^{b} \pm 5.8$	$30.0^{bc} \pm 0.0$	$33.3^{bcd} \pm 3.3$
Ch 3	$0.0^{b} \pm 0.0$	$6.7^{b} \pm 3.3$	$6.7^{b} \pm 3.3$	$10.0^{bc} \pm 0.0$	$13.3^{de} \pm 3.3$
Ch 4	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$16.7^{b} \pm 12.0$	$26.7^{bc} \pm 12.0$	$30.0^{bcd} \pm 10.0$
Ch 1-Ag	$0.0^{b} \pm 0.0$	$6.7^{b} \pm 3.3$	$13.3^{b} \pm 6.7$	$16.7^{bc} \pm 6.7$	$20.0^{cde} \pm 5.8$
Ch 1-Cu	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$	$16.7^{b} \pm 3.3$	$20.0^{bc} \pm 0.0$	$36.7^{bc} \pm 3.3$
Ch 1-Ni	$6.7^{b} \pm 3.3$	$23.3^{b} \pm 3.3$	$26.7^{b} \pm 6.7$	$30.0^{bc} \pm 5.8$	$93.3^{a} \pm 3.3$
Ch 1-Hg	$13.3^{a} \pm 3.3$	$50.0^{a} \pm 11.6$	$60.0^{a} \pm 5.8$	$80.0^{a} \pm 5.8$	$83.3^{a} \pm 3.3$
F-ration	9.0	10.0	5.6	11.5	34.7
df	8, 18	8, 18	8, 18	8, 18	8, 18
P-value	< 0.0001	< 0.0001	0.001	< 0.0001	< 0.0001

Ch 1 – chitosan of 2.27×10^5 g/mol; Ch 2 – chitosan of 3.60×10^5 g/mol; Ch 3 – chitosan of 5.97×10^5 g/mol; Ch 4 – chitosan of 9.47×10^5 g/mol; Data are expressed as mean percentages ± SE of three replicates; Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test; df – degree of freedom; *P*-value – significance of the *F* ratio

Table 3. Growth inhibition and antifeedant activity (%) of chitosan compounds against third-instar larvae of *S. littoralis* by feeding on 4 g (a.i) chitosan/kg diet

Treatment	Growth inhibition (%) through 7 days of feeding					Antifeedant (%) ± SE after 7 days
	1	2	3	4	7	
Control	0	0	0	0	0	0.0^{d} ± 0.0
Ch 1	37.4	35.5	54.8	64.9	77.8	76.0^{ab} ± 2.5
Ch 2	17.1	20.2	36.7	36.2	30.3	37.0^{c} ± 12.5
Ch 3	28.7	12.3	28.8	28.2	6.9	2.3^{d} ± 0.9
Ch 4	6.7	6.4	31.9	30.5	21.6	35.4^{c} ± 6.3
Ch 1-Ag	13.6	8.9	20.9	26.7	0.3	0.2^{d} ± 7.4
Ch 1-Cu	31.3	43.2	47.8	61.0	54.5	61.6^{b} ± 4.4
Ch 1-Ni	44.1	67.3	75.3	88.3	97.3	90.2^{a} ± 5.7
Ch 1-Hg	65.9	82.9	87.0	90.3	96.2	86.8^{a} ± 7.6
F-ratio						32.6
df						8, 18
P-value						< 0.0001

Ch 1 – chitosan of 2.27×10^5 g/mol; Ch 2 – chitosan of 3.60×10^5 g/mol; Ch 3 – chitosan of 5.97×10^5 g/mol; Ch 4 – chitosan of 9.47×10^5 g/mol; Data are expressed as mean percentages based on 3 replicates per tested compound; Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test; df – degree of freedom; *P*-value – significance of the *F*-ratio

chitosan of 2.27×10^5 g/mol (Ch 1) and its metal complexes with Cu, Ni, and Hg were the most active compounds (76, 62, 90, and 87% antifeedant activity, respectively).

Insecticidal activity of chitosans and chitosan-metal complexes against *A. nerii*

The aphicidal activity of chitosans of different molecular weights and chitosan-metal complexes against the oleander aphid *A. nerii* by leaf dip and systemic action bioassays is shown in Table 4. Chitosans of 3.60×10^5, 5.97×10^5 and 9.47×10^5 g/mol (Ch 2, Ch 3, and Ch4, respectively) exhibited high aphicidal activity among the four different molecular weights of chitosan in leaf-dip bioassay after 24 h of treatment with 48, 49, and 46% mortalities at 1000 mg/l, respectively (Table 4). However, chitosan of 2.27×10^5 g/mol (Ch 1) was the most active compound after 48 h (71, 73, and 84% at 250, 500, and 1000 mg/l, respectively) compared to 5% in the control of water and 10% in the control of aqueous acetic acid (0.5%). When low molecular weight chitosan, Ch 1 of 2.27×10^5 g/mol, was linked with metals of Ag(I), Cu(II), Ni(II), and Hg(II), the aphicidal activity did not increase significantly compared to the unmodified chitosans. However, chitosan-Ni was the most significantly active among the chitosan-metal complexes after 24 and 48 h of the treatment except that obtained with chitosan-Ag at 1000 mg/l (85% mortality after 48 h).

The aphicidal activity by systemic bioassay as shown in Table 4 confirmed that all compounds have a systemic effect after 48 h at 250, 500, and 1000 mg/l (df = 25, 52; $F = 223$; $P < 0.0001$). This indicates that chitosan compounds are primarily translocated in the plant phloem, which passively transports mainly water in an acropetal, i.e. upward movement. After the chitosan molecule moved into the plant, the aphids died and the treatments protected the plant. Chitosans of 2.27×10^5, 3.60×10^5, and 5.97×10^5 g/mol (Ch 1, Ch 2, and Ch 3, respectively) showed the significantly highest efficacy at all concentrations tested with 96, 87, and 100% mortalities, respectively, at 1000 mg/l. However high molecular weight, Ch 4 of 3.60×10^5 g/mol, showed moderate activity with mortalities of 49, 68, and 74% at 250, 500, and 1000 mg/l, respectively. Among chitosan-metal complexes, chitosan-Cu was significantly the most active compound with 70, 73, and 94% mortalities at 250, 500, and 1000 mg/l, respectively. However, chitosan-Hg was significantly the least active one with 44, 48, and 52% mortalities at 250, 500, and 1000 mg/l, respectively.

Table 4. Insecticidal activity (mortality % ± SE) of different molecular weights chitosan and chitosan-metal complexes against *A. nerii* by leaf dip method and systemic effect method after 48 hours

Treatment	Concentration (mg/l	Leaf dip method		Systemic effect method
		24 h	48 h	48 h
CW	0	$0.0^{l} \pm 0.0$	$4.7^{k} \pm 4.8$	$12.9^{l} \pm 1.8$
CA	0	$6.7^{kl} \pm 3.3$	$10.0^{k} \pm 0.0$	$19.0^{k} \pm 2.4$
Ch 1	250	$19.4^{hi} \pm 0.6$	$70.9^{bcde} \pm 0.9$	$71.7^{de} \pm 1.0$
	500	$29.9^{fgh} \pm 3.2$	$73.1^{bcd} \pm 0.2$	$75.4^{d} \pm 1.4$
	1000	$32.2^{efg} \pm 2.2$	$84.4^{a} \pm 1.1$	$95.7^{b} \pm 0.0$
Ch 2	250	$22.9^{ghi} \pm 2.2$	$52.6^{hi} \pm 1.4$	$53.6^{h} \pm 0.9$
	500	$46.0^{abcde} \pm 3.9$	$55.0^{gh} \pm 1.3$	$69.7^{def} \pm 0.6$
	1000	$48.0^{abcde} \pm 3.5$	$64.0^{defgh} \pm 0.7$	$86.5^{c} \pm 0.2$
Ch 3	250	$45.4^{abcdef} \pm 1.6$	$69.9^{bcdef} \pm 0.7$	$57.9^{g} \pm 1.2$
	500	$45.6^{abcdef} \pm 2.9$	$73.9^{bcd} \pm 0.6$	$85.6^{c} \pm 0.7$
	1000	$49.2^{abcd} \pm 3.2$	$80.3^{ab} \pm 2.7$	$100.0^{a} \pm 0.0$
Ch 4	250	$37.3^{cdef} \pm 1.9$	$58.8^{fgh} \pm 3.4$	$49.3^{h} \pm 2.0$
	500	$42.1^{bcdef} \pm 4.1$	$60.4^{efgh} \pm 3.6$	$67.7^{ef} \pm 0.3$
	1000	$46.5^{abcde} \pm 4.9$	$64.0^{defgh} \pm 1.5$	$73.8^{d} \pm 2.4$
Ch 1-Ag	250	$34.9^{cdefg} \pm 0.8$	$55.2^{gh} \pm 2.6$	$42.4^{j} \pm 3.0$
	500	$35.6^{cdefg} \pm 2.2$	$64.4^{def} \pm 2.2$	$58.7^{g} \pm 0.7$
	1000	$36.1^{cdefg} \pm 1.4$	$85.1^{a} \pm 1.9$	$65.1^{f} \pm 1.6$
Ch 1-Cu	250	$38.6^{cdef} \pm 1.4$	$63.8^{defgh} \pm 3.8$	$69.7^{def} \pm 0.5$
	500	$38.9^{cdef} \pm 5.6$	$67.7^{cdef} \pm 3.6$	$73.5^{de} \pm 0.8$
	1000	$43.1^{bcdef} \pm 3.4$	$71.8^{bcde} \pm 1.3$	$94.0^{b} \pm 1.2$
Ch 1-Ni	250	$54.8^{ab} \pm 2.4$	$72.6^{bcd} \pm 1.2$	$43.6^{ij} \pm 2.6$
	500	$55.6^{ab} \pm 5.6$	$77.8^{abc} \pm 2.8$	$50.0^{h} \pm 1.3$
	1000	$58.9^{a} \pm 4.8$	$81.3^{ab} \pm 4.0$	$83.1^{c} \pm 1.8$
Ch 1-Hg	250	$12.5^{ik} \pm 0.0$	$45.8^{i} \pm 4.2$	$44.1^{ij} \pm 0.7$
	500	$33.6^{defg} \pm 2.2$	$63.1^{defgh} \pm 1.9$	$48.2^{hi} \pm 1.9$
	1000	$50.0^{abc} \pm 5.0$	$73.5^{bcd} \pm 2.3$	$51.9^{h} \pm 0.9$
F-ratio		21.1	59.8	222.7
df		25, 52	25, 52	25, 52
P-value		< 0.0001	< 0.0001	< 0.0001

CW – control water; CA – control acid (05%, v/v, aqueous acetic acid); Ch 1 – chitosan of 2.27×10^5 g/mol; Ch 2 – chitosan of 3.60×10^5 g/mol; Ch 3 – chitosan of 5.97×10^5 g/mol; Ch 4 – chitosan of 9.47×10^5 g/mol; Data are averages ± SE of three replicates; Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test; df – degree of freedom, *P*-value – significance of the *F* ratio

DISCUSSION

Chemical pesticides provide the primary means for controlling agricultural insect pests. Continuous use of these compounds has faced two major obstacles: increasing public concern regarding the contamination of perishables with pesticide residues, and proliferation of resistance in the pest populations. The ultimate aim of recent research in this area has been the development and evaluation of various alternative control strategies to reduce dependence on synthetic pesticides. Recently, the exploitation of a bioactive chitosan polymer and its derivatives to control agricultural pests has received more attention. In the present study, complexes of this polymer with the most active metal ions were tested as alternatives to synthetic pesticides in controlling the economically most important agricultural pests. Farmers have used metal compounds in phytosanitary treatments for

more than a century; however, it has recently been suggested that plants absorb high concentrations of metals from the substrate as a self-defence mechanism against pathogens and herbivores (Reeves *et al*. 1981; Poschenrieder *et al*. 2006).

Chitosan has been shown to have the best chelating properties among natural polymers (Varma *et al*. 2004). Amino groups distributed on the chitosan skeleton are responsible for the metal-complex formation where nitrogen is a donor of electron pairs, although hydroxyl groups can also participate in sorption. The mechanism of combining these reactive groups with ions of metals is much differentiated and can depend on the ion type and pH of the solution. The formation of chitosan-metal complexes could be also described based on Lewis acid-base theory: metal ions acting as the acid are the acceptor of a pair of electrons given by chitosan acting as the base. Based on this information, the reasonable structure of chitosan-metal complexes is shown in Figure 2 according to the hypothesis of Wang *et al*. (2004). As shown, metal ion like a bridge connected one or more chains of chitosan through interacting with -OH and -NH_2 groups.

Previous studies on the biological activity of chitosan and its derivatives against *S. littoralis* reported that the unmodified chitosan showed low insecticidal activity against larvae of *S. littoralis*, but its chemical modification led to an increase in the activity (Badawy *et al*. 2005; Rabea *et al*. 2005, 2006; Badawy 2008). Bioassays with 17 derivatives of *N*-(alkyl) chitosans at a rate of 5 g/kg diet demonstrated that *N*-(propyl) chitosan, *N*-(undecanyl) chitosan and *N*-(3-phenylpropyl) chitosan strongly inhibited larval weight gain in

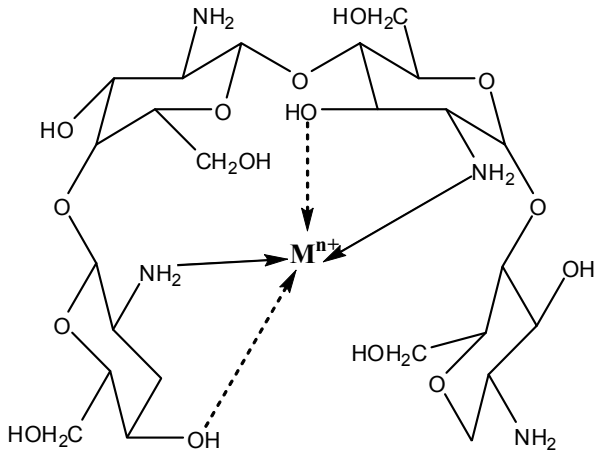

Figure 2. The reasonable structure of chitosan-metal complexes according to Wang *et al*. (2004)

S. littoralis, with respective reductions of 77 and 65% after 4 days of feeding (Rabea *et al*. 2006). Moreover, *N*-(benzyl) chitosan derivatives had significant insecticidal activity at 5 g/kg diet. Among 16 derivatives, *N*-(*p*-isopropylbenzyl) chitosan, *N*-(2-chloro-6-fluorobenzyl) chitosan, and *N*-(*o*-nitrobenzyl) chitosan caused significant mortalities of 46, 100 and 46%, respectively (Rabea *et al*. 2005). In addition, the activity of chitosan and oligo-chitosan against several plant-feeding insects was studied previously (Zhang *et al*. 2003). The insecticidal activity of chitosan against the diamondback moth, *Plutella xylostella* L., was higher, with 72% mortality. The insecticidal activity of chitosan against another caterpillar, *Helicoverpa armigera* Hubner, was 38 and 40% after 24 and 72 h, respectively.

The present study indicated that the incorporation of chitosan compounds into the diet reduced the mean weight gain of *S. littoralis* larvae and chitosan-Ni and chitosan-Hg showed high larval mortality and they were the most active among chitosan-metal complexes in the inhibition of larval growth. This finding is in agreement with the recent studies by Coleman *et al*. (2005) and Jhee *et al*. (2006), who determined the toxicity thresholds of different metals in the diamondback moth fed with artificial diets containing metal salts. They have revealed that Cd, Mn, Ni, Pb, and Zn can be toxic to this folivore even at concentrations below accumulator levels.

The conventional technology using synthetic insecticides has not been effective enough against numerous homopterous pests such as aphids because these pests generally feed on the underside of foliage or within the plant canopy. Their small size, short life cycle, and high fecundity often permit dramatic rises in population sizes. Also, rapid differentiation of populations into insecticide-resistant strains necessitates increasing insecticidal treatments, which are economically and ecologically undesirable (Puterka *et al*. 1988). Therefore, an effective way to delay resistance to insecticides and still maintain insect population densities below the economic threshold is to reduce the use of pesticides with the integration of other control strategies (Tabashnik 1986). Interestingly in the present study, the activity of chitosan compounds against *A. nerii* by leaf dip and systemic methods is good. The result confirmed that aphids feeding on treated leaves for 24 and 48 h were significantly affected; this suggests that oral uptake is essential for the aphid control. Comparing leaf dipping

and systemic action techniques for aphids, the former is easy to use. The aphicidal activity that was obtained in the present study is in agreement with previous studies that have demonstrated both aversion responses by invertebrate herbivores towards plants that hyperaccumulate metals (Jhee *et al*. 1999; Behmer *et al*. 2005), and the negative consequences of continual feeding on such plants for growth and survival of the herbivore (Martens & Boyd 1994; Boyd & Moar 1999).

Aphicidal activities of chitosan diethyl phosphate and chitosan ethyl carbamate, at different concentrations, have been evaluated against the green peach aphid (*Myzus persicae*) and compared with imidacloprid (Cabrera *et al*. 2002). The results showed that chitosan diethyl phosphate at 0.5% showed the highest lethal activity compared to imidacloprid. In pot assays, chitosan diethyl phosphate and imidacloprid showed a systemic effect in sugar beet plants. The systemic effect was not observed using chitosan and chitosan carbamate suggesting that the insecticidal activity is due to the hydrolysis of the diethyl phosphate moiety (Cabrera *et al*. 2002; Cardenas *et al*. 2002; Casals *et al*. 2002; Placencia *et al*. 2003). Chitosan also exhibits insecticidal activity against various aphids at a range of concentrations from 600 to 6000 mg/l. For example, chitosan had a very high insecticidal activity against *Hyalopterus pruni* (Goffroy) on flowers, giving corrected mortalities between 93 and 99%. In addition, chitosan showed a 70–80% insecticidal activity against *Rhopalosiphum padi* (L.), *Metopolophium dirhodum* (Walker), and *Aphis gossypii* (Glover), while *Sitobion avenae* (Fabricius) and *M. persicae* (Sulzer) showed a lower susceptibility to chitosan (Zhang *et al*. 2003).

CONCLUSION

The importance of this work is to use chitosan as biologically active compound against some economic pests. Chitosan at different molecular weights and chitosan complexes with metals of Ag(I), Cu(II), Ni(II), and Hg(II) showed good insecticidal activity against larvae of cotton leafworm *S. littoralis* and oleander aphid *A. nerii*. *S. littoralis* larvae fed chitosan-Ni and chitosan-Hg complexes halted feeding after 3 days, but the mechanism of action remains unknown. These results suggested that some of these compounds have the potential of becoming alternatives for plant protection instead of some harmful pesticides. In addition, they may be introducing a novel mode of action on insects, so that development of resistance is not likely to happen.

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