Nematicidal Activity of a Biopolymer Chitosan at Different Molecular Weights against Root-Knot Nematode, *Meloidogyne incognita*

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Abstract

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The nematicidal activity of four molecular weights (2.27×10^5, 3.60×10^5, 5.97×10^5, and 9.47×10^5 g/mol) of a biopolymer chitosan was assayed against the root-knot nematode, *Meloidogyne incognita*, *in vitro* and in pot experiments. In laboratory assays, the nematode mortality was significantly influenced by exposure times and chitosan molecular weight. Low molecular weight chitosan (2.27×10^5 g/mol) was the most effective in killing the nematode with EC_{50} of 283.47 and 124.90 mg/l after 24 and 48 h of treatment, respectively. In a greenhouse bioassay, all the compounds mixed in soil at one- and five-fold concentrations of the LC_{50} value significantly reduced population, egg mass, and root galling of tomato seedlings compared with the untreated control. In general, the nematicidal activity of these compounds was increased dramatically with a decrease in the molecular weight. The results suggest that the chitosan at low molecular weight may serve as a natural nematicide.

Keywords: biopolymer chitosan; different molecular weight; mematode mortality

Nematodes are the most numerous Metazoa on the Earth and they are essentially aquatic animals. They are either free-living or parasites of plants and animals (Cobb 1915; de Ley & Blaxter 2002). The majority of plant-parasitic nematodes are root feeders, completing their life cycles in the root zone while they are found in association with most plants. Some are endoparasitic, living, and feeding within the tissue of roots, tubers, buds and seeds (Sasser 1990). Direct feeding of nematodes can drastically decrease a plant's uptake of water and nutrients. Plant-parasitic nematodes have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination (Ploeg & Stapleton 2001). Root-knot nematodes, *Meloidogyne* spp., are common pathogens that parasitise vegetables and other crops and cause significant yield reductions worldwide (Sasser 1980, 1990; Sasser & Freckman 1987; Jones *et al.* 1991; Pajovic *et al.* 2007). They disrupt the physiology of the plant and may reduce crop yield and product quality and, therefore, are of great economic importance. Four major species, namely *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita,* and *Meloidogyne javanica,* have been reported to infect tomatoes in the tropics (Sasser 1979). These species cause galls or root-knots on infected plants. Other symptoms include stunted growth, wilting and poor fruit yield. Infection by *M. incognita* can increase root weight and decrease shoot weight (Fortnum *et al.* 1991).

Prevention and control of such pests will remain an important objective of most researches. Today, plant-parasitic nematodes are controlled by cultural practices, chemical nematicides, and by the grow-

ing of resistant cultivars (Bridge 1996; Barker & Koenning 1998; Curto *et al.* 2006). Chemical control is expensive and is economically viable only for high-value crops and creates a potential hazard to the environment and human health. Therefore, alternative nematode control methods or less toxic nematicides need to be developed (Noling & Becker 1994; Ploeg 2007). One way of searching for such nematicidal compounds is to screen naturally occurring compounds in plants (Halbrendt 1996; Oka *et al.* 2000; Wiratno *et al.* 2009).

At present, chitin and chitosan have useful nematistatic and nematicidal activity for agricultural and horticultural applications by admixing nematicidally effective amounts to a plant growth medium (Cardenas-Trivino *et al.* 1989; Vasyukova *et al.* 2001; Aboud *et al.* 2002; Kalaiarasan *et al.* 2006). The complex also provides a source of nitrogen in slow-release form, making it particularly suitable for combination with fertilisers and soil conditioners. The use of chitosan as a new line developed and improved to be an important tool in the integrated pest management (IPM) programs and as a safe method for human health and environment.

Chitosan is a well-studied linear polysaccharide, polymerised from the monomers 2-acetoamido-2-deoxy-β-D-glycopyranose (GlcNac) and 2-amino-2-deoxy-β-D-glycopyranose (GlcN), which contains a higher portion of GlcN causing the solubility of chitosan in aqueous media and it is produced from crustaceous shell waste (No & Meyers 1997). Chitosan and its derivatives have been used in a wide variety of applications (Rabea *et al.* 2003), but the properties and effectiveness of these materials are dependent upon their molecular size. Chitosan is, like other polysaccharides, susceptible to a variety of degradation mechanisms, including oxidative-reductive, free radical depolymerisation, acid, alkaline, and enzymatic-catalysed hydrolysis. With the knowledge that the biological activity of chitosan is dependent on the molecular weight of the compound and the microorganism species being targeted, the objectives of this study were to (*i*) determine the effect of different molecular weights of a biopolymer chitosan on a plant-parasitic nematode, root-knot nematode *M. incognita*, (*ii*) quantify lethal concentration (LC_{50}) values of those chitosans that have a potential for application as nematicidal natural compounds for nematode suppression, and (*iii*) the compounds are subsequently tested in a greenhouse experiment to evaluate their *in vivo* effectiveness as a mulch to control the nematode attacking the roots of tomato plants.

MATERIAL AND METHODS

Material. Four molecular weights of acid-soluble chitosans were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Degrees of deacetylation were 89, 85, 81, and 82%, respectively. All chitosans were in a powder form and prepared from crab shells. Acetic acid, sodium acetate, and sodium nitrite ($NaNO_2$) were used without further purification, and all reagents used were of analytical reagent grade. Vydate®10% G (oxamyl), [*N*,*N*-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio) acetamide] (Du Pont Agro Hellas S.A., Cairo, Egypt).

Viscosity measurement and determination of chitosan molecular weight. The viscosity of chitosan solutions in CH_3COOH/CH_3COONa buffer (0.5:0.2 mol/l, respectively) was measured with an Ubbelohde Viscometer (capillary section size 0.7 mm) immersed in a constant temperature bath at 25.0 ± 0.1°C. The stock solutions were prepared and diluted to yield lower concentrations prepared by adding the appropriate amount of the buffer to the stock solutions. The capillary was filled with 25 ml of sample and equilibrated in a water bath to maintain the appropriate temperature. The sample was passed through the capillary once before the running time was measured and each sample was measured three times. The running times of the solution and solvent were used to calculate relative viscosity, specific viscosity, and reduced viscosity as follows:

$$\text{Relative viscosity } (\eta_{rel}) = t_{ch}/t_{sol}$$

$$\text{Specific viscosity } (\eta_{sp}) = (\eta_{rel})^{-1}$$

$$\text{Reduced viscosity } (\eta_{red}) = \eta_{sp}/c$$

where:

t_{ch} – running time of the chitosan solution
t_{sol} – running time of the solvent
c – chitosan concentration in g/dl

Intrinsic viscosity, defined as $[\eta] = C(\eta_{red})_c = 0$, was obtained by extrapolating the reduced viscosity versus concentration data to zero concentration and the intercept on the ordinate is the intrinsic

viscosity (Rinaudo & Domard 1989; Allan & Peyron 1995a,b). The molecular weight was calculated based on the Mark-Houwink-Sakurada equation ($\eta = KM^a$) as described by Flory (1953) and Tanford (1961), where η is the intrinsic viscosity, K and a are viscometric parameters depending on the solvent. For a chitosan dissolved in 0.5M CH_3COOH/0.2M CH_3COONa as in the present study the K and a were found to be 3.5×10^{-4} and 0.76, respectively, according to Wang *et al.* (1991).

Collection of assay nematode. The eggs of the root-knot nematode, *M. incognita* (Tylenchida: Heteroderidae), were isolated from infested roots of eggplant (*Solanum melongena* L.) collected from Alexandria Governorate fields while they showed typical symptoms of the root-knot nematode disease. Sodium hypochlorite (NaOCl) was used for the isolation of nematode eggs from root galls according to Hussey and Barker (1973) and then they were passed through 200 and 400 mesh sieves to obtain free eggs directly before carrying out the experiments and then they were put in the incubator at 27°C for 72 hours. The suspension of two-days-old second-stage larvae (juveniles) of *M. incognita* was passed through a 325 mesh sieve and then the retained larvae were collected on the sieve by backwashing into a 250-ml beaker, using tap water. The larvae per 1 ml were counted under a microscope which showed 110 larvae/1 ml.

In vitro study. The suspension (1 ml) of freshly hatched juveniles (100–120 juveniles) and 1 ml of each concentration of the tested chitosan compound were transferred separately into glass cavity blocks and kept at a room temperature of 27 ± 2°C. There were three replicates of each treatment and juvenile mortality was recorded after 24 and 48 hours. The larvae in the suspension were distributed in glass vials (10 ml vol.) after having been counted and each vial contained 1 ml of the suspension (almost 120 larvae), while the total volume in the glass vials was 3 ml. Chitosan compounds were prepared in 0.25% (v/v) aqueous acetic acid, then diluted to 0.01% aqueous acetic acid. The chitosan was used in four different molecular weights and in different concentrations which were 125, 250, 500, and 1000 mg/l, then it was used to treat the larvae in the glass vials which were kept under laboratory conditions at 27 ± 2°C. There were three replicates of each treatment and nematodes were counted after 24 and 48 h of the treatment using a stereomicroscope under 100× magnification.

***In vivo* study.** An experiment was carried out under greenhouse conditions to evaluate the efficacy of chitosan compounds with different molecular weights in the control of *M. incognita*, using tomato (*Lycopersicon esculentum* Mill.) plants cv. Super strain B as a host plant. The tomato plants were cultivated in black sheet (10 cm in diameter and 20 cm in depth) that was filled with 600 g soil (1:1, clay:sand). The nematode eggs were applied at the rate of 5000 eggs/replicate. Each treatment was replicated three times and every replicate contained one seedling. After 2 days, the nematicide (oxamyl) was applied according to the recommended dose, while the chitosan compounds were used at two rates (one- and five-fold LC_{50} value after 24 h of the *in vitro* experiment). All treatments were applied once after inoculation within three days as a soil drench and the total experimental time was 60 days, then the plants were lifted and root galls and egg masses were determined. The fertilisation of plants was carried out every two weeks with NPK (20-20-20) by a soil drench. The irrigation was performed every two days. Egg masses and gall number per root system and number of juveniles per 200 g soil were evaluated. The roots were stained in an aqueous solution of phloxine B stain (0.15 g/l water) for 15 min and then washed with running tap water to remove the residual stain and to detect the presence of nematode egg masses (Holbrook *et al.* 1983). In addition, the averages of shoot, root length (in cm) and shoot and root weight (in g) of plants were measured.

Statistical analysis. Statistical analysis was performed using SPSS 12.0 software program (Statistical Package for Social Sciences, Chicago, USA). The mortality rates of the nematodes in the exposure groups were corrected for mortality in the solvent controls using Abbott's formula (Abbot 1925). The corrected mortalities were plotted against the concentrations and fitted using SPSS software to determine the LC_{50} according to the probit analysis (Finney 1971). The 95% confidence limits for the range of LC_{50} values were determined by the least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration. The data of *in vivo* experiments were analysed by one-way analysis of variance (ANOVA). Mean separations were performed by the Student-Newman-Keuls (SNK) test and differences at $P < 0.05$ were considered as significant.

RESULTS

Characterization of chitosan compounds

Four chitosan compounds with different 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 were used in the present study with degrees of deacetylation of 89, 85, 81, and 82%, respectively. The viscosity measurement performed with the purified chitosan samples allowed the determination of their intrinsic viscosities and viscosity average molecular weights. In the present study, the molecular weights of 2.27×10^5, 3.60×10^5, 5.97×10^5, and 9.47×10^5 g/mol were found with the native chitosan compounds (Table 1). The resulting values allow to qualify four different chitosan compounds when their viscosity average molecular weights are compared. The curves relating reduced viscosities and chitosan concentrations for the purified polymers (Figure 1) show that all experimental points are very well aligned along straight lines ($r > 0.98$).

Table 1. Characterisation of chitosan compounds of different molecular weights

Compound	DA (%)	DDA (%)	η	MW (g/mol)
Ch 1	11	89	4.12	2.27×10^5
Ch 2	15	85	5.84	3.60×10^5
Ch 3	19	81	8.58	5.97×10^5
Ch 4	18	82	12.19	9.47×10^5

Ch 1 – 2.27×10^5 g/mol; Ch 2 – 3.60×10^5 g/mol; Ch 3 – 5.97×10^5 g/mol; Ch 4 – 9.47×10^5 g/mol; DA – degree of acetylation; DDA – degree of deacetylation; η – intrinsic viscosity; MW – molecular weight

In vitro assays

The effects of chitosans of different molecular weights on two-days-old second-stage larvae (juveniles) of *M. incognita* were examined (Table 2). It can be noticed that the nematicidal activity was increased dramatically with a decrease in the molecular weight. Chitosan of 2.27×10^5 g/mol (Ch 1) exerted significantly prominent nematicidal activity with LC_{50} of 283.47 and 124.90 mg/l after 24 and 48 h, respectively. However, chitosan of 9.47×10^5 g/mol (Ch 4) was the least active one with LC_{50} of 523.00 and 260.08 mg/l after 24 and 48 h, respectively.

Pot experiments

In pot experiments, the four molecular weights of chitosan at one- and five-fold concentrations of LC_{50} value were significantly effective in reducing the nematode population, root galling and egg mass compared with the control after two months of a single application (Table 3). The experiments revealed that the total number of live nematodes on roots of tomato plants treated with oxamyl, as a specific nematicide, was significantly the lowest when it reduced the population to 358.67 larvae/200 g soil (89.05% reduction) compared with 3276.67 larvae/200 g soil in the control. Chitosans of 2.27×10^5 g/mol (Ch 1) and 3.60×10^5 g/mol (Ch 2) were significantly the most effective in the reduction of nematode population among the others and there was no significant difference between them at treatment with fivefold LC_{50} value. At the end of the experiment, 1029.00 and 1057 larvae/200 g soil were counted with Ch 1 and Ch 2,

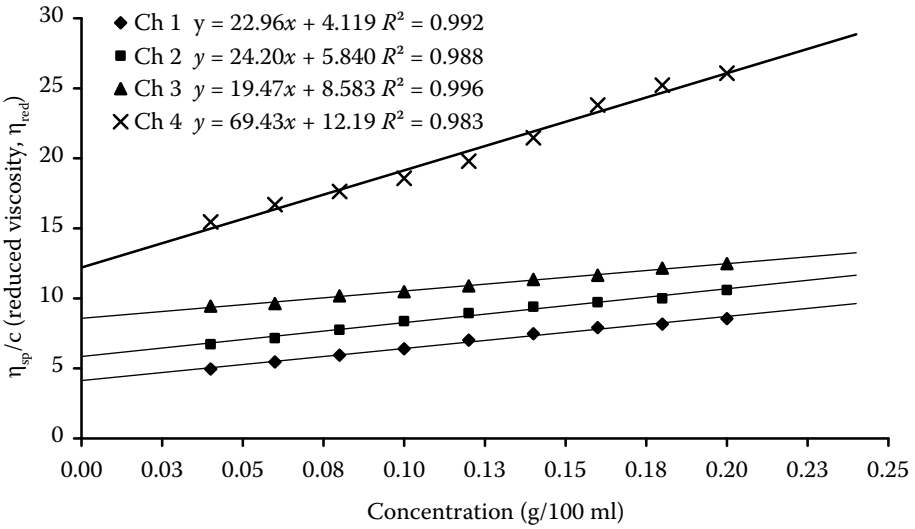

Figure 1. Curves of reduced viscosity (η_{red}) against concentrations of chitosan compounds of different molecular weights

Table 2. The *in vitro* nematicidal activity of chitosans of different molecular weights against *M. incognita*

Compound	LC_{50} (mg/l)	95% confidence limits (mg/l)		Slope ± SE	Intercept of regression line ± SE	χ^2
		lower	upper			
After 24 h						
Ch 1	283.47	232.19	338.79	1.69 ± 0.21	−4.14 ± 0.53	1.79
Ch 2	395.83	331.97	476.67	1.72 ± 0.21	−4.49 ± 0.53	0.80
Ch 3	429.23	360.86	521.22	1.72 ± 0.21	−4.53 ± 0.54	3.32
Ch 4	523.00	435.00	653.75	1.66 ± 0.21	−4.52 ± 0.55	3.33
After 48 h						
Ch 1	124.90	90.18	155.80	1.92 ± 0.25	−4.02 ± 0.61	3.78
Ch 2	181.16	39.90	302.34	2.41 ± 0.26	−5.44 ± 0.63	5.43
Ch 3	215.12	57.93	381.31	2.66 ± 0.26	−6.20 ± 0.63	6.88
Ch 4	260.08	206.07	316.23	1.53 ± 0.20	−3.70 ± 0.52	2.42

Ch 1 – 2.27×10^5 g/mol; Ch 2 – 3.60×10^5 g/mol; Ch 3 – 5.97×10^5 g/mol; Ch 4 – 9.47×10^5 g/mol

Table 3. The effect of soil treatment with chitosans of different molecular weights on population, egg mass, and root galling of tomato seedlings cultivated in soil infested with *M. incognita*

Treatments	Population (larvae/200 g soil) ± SE	Reduction (%)	Number of galls ± SE	Reduction (%)	Number of egg mass/root ± SE	Reduction (%)
Control	3276.67^{a} ± 49.10	0.00	450.00^{a} ± 2.65	0.00	354.67^{a} ± 3.53	0.00
Oxamyl	358.67^{i} ± 4.67	89.05	63.67^{h} ± 2.03	85.85	115.33^{h} ± 2.91	67.48
Ch 1 (1-fold)	1565.33^{e} ± 5.33	52.23	133.67^{e} ± 2.03	70.30	156.33^{d} ± 3.18	55.92
Ch 1 (5-fold)	1029.00^{h} ± 5.51	68.60	75.00^{g} ± 2.89	83.33	121.67^{fg} ± 1.76	65.70
Ch 2 (1-fold)	1661.67^{d} ± 10.93	49.29	191.33^{c} ± 2.40	57.48	170.33^{c} ± 3.76	51.97
Ch 2 (5-fold)	1057.33^{h} ± 2.67	67.73	96.67^{f} ± 2.40	78.52	127.67^{f} ± 1.86	64.00
Ch 3 (1-fold)	1766.67^{c} ± 19.22	46.08	194.33^{c} ± 2.33	56.81	177.00^{bc} ± 1.73	50.09
Ch 3 (5-fold)	1182.33^{g} ± 24.33	63.92	103.33^{f} ± 2.40	77.04	143.00^{e} ± 2.08	59.68
Ch 4 (1-fold)	2042.33^{b} ± 11.35	37.67	249.00^{b} ± 4.04	44.67	182.33^{b} ± 1.20	48.59
Ch 4 (5-fold)	1499.67^{f} ± 5.78	54.23	179.00^{d} ± 4.04	60.22	157.00^{d} ± 2.52	55.73
F	1589.82		1647.17		707.18	
df	9, 20		9, 20		9, 20	
P	< 0.0001		< 0.0001		< 0.0001	

Ch 1 – 2.27×10^5 g/mol; Ch 2 – 3.60×10^5 g/mol; Ch 3 – 5.97×10^5 g/mol; Ch 4 – 9.47×10^5 g/mol; data are means ± SE of three replicates; values within a column bearing the same superscript are not significantly different ($P \leq 0.05$) according to the Student-Newman-Keuls (SNK) test; *F* – *F* ratio, df – degrees of freedom; *P* – *P* value (significance of the *F* ratio)

respectively, at the treatment with the fivefold LC_{50} value compared with 3276.67 larvae/200 g soil in the control. Roots of tomato plants grown in the soil treated with chitosan compounds had very few galls especially with Ch 1 at the fivefold LC_{50} value (75.00 galls/plant, 83.33% reduction), while control plants were heavily galled (450 galls/root). In addition, all the treatments were significantly effective in the reduction of egg mass compared with the untreated plants and also chitosan with the lowest molecular weight was the most active one (121.67 egg mass/plant compared with 354.67 egg mass/plant in the control). Generally, it can be noticed that the nematicidal activity was increased dramatically with a decrease in the molecular weight. Moreover, there was no plant mortality during the experiment while untreated plants were significantly affected by nematodes.

Chitosan treatments significantly ($P \leq 0.05$) enhanced the seedling growth to a different ex-

Table 4. The effect of chitosan compounds of different molecular weights on the length and weight of tomato seedlings cultivated in soil infested with *M. incognita*

Treatments	Shoot length (cm) ± SE	Root length (cm) ± SE	Total fresh weight (g/plant) ± SE	Total dry weight (g/plant) ± SE
Control	46.33^{d} ± 1.03	21.67^{cd} ± 0.88	18.30^{d} ± 0.96	2.53^{e} ± 0.12
Oxamyl	53.67^{c} ± 0.51	32.33^{a} ± 1.33	38.83^{bc} ± 2.49	5.47^{ab} ± 0.15
Ch 1 (1-fold)	67.33^{ab} ± 1.03	36.00^{a} ± 3.00	46.10^{a} ± 2.26	5.10^{b} ± 0.17
Ch 1 (5-fold)	71.33^{a} ± 1.80	31.83^{a} ± 0.93	48.67^{a} ± 2.46	5.71^{a} ± 0.23
Ch 2 (1-fold)	64.33^{ab} ± 0.64	24.00^{bc} ± 1.53	37.97^{bc} ± 0.61	4.17^{c} ± 0.13
Ch 2 (5-fold)	70.33^{ab} ± 2.18	25.33^{b} ± 0.88	40.67^{b} ± 1.42	4.63^{c} ± 0.13
Ch 3 (1-fold)	62.00^{b} ± 1.92	19.83^{c} ± 1.59	34.07^{bc} ± 0.77	3.53^{d} ± 0.12
Ch 3 (5-fold)	67.67^{ab} ± 1.28	22.50^{cd} ± 0.76	38.43^{bc} ± 0.79	4.40^{c} ± 0.12
Ch 4 (1-fold)	62.33^{b} ± 1.28	19.17^{c} ± 0.44	32.50^{c} ± 0.45	3.47^{d} ± 0.17
Ch 4 (5-fold)	64.67^{ab} ± 1.41	21.83^{cd} ± 1.30	34.57^{bc} ± 1.92	3.70^{d} ± 0.06
F	16.20	24.74	26.87	46.21
df	9, 20	9, 20	9, 20	9, 20
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Ch 1 – 2.27×10^5 g/mol; Ch 2 – 3.60×10^5 g/mol; Ch 3 – 5.97×10^5 g/mol; Ch 4 – 9.47×10^5 g/mol; data are means ± SE of three replicates; values within a column bearing the same superscript are not significantly different ($P \leq 0.05$) according to the Student-Newman-Keuls (SNK) test; *F* – *F* ratio; df – degrees of freedom; *P* – *P* value (significance of the *F* ratio)

tent over the controls. Shoot and root length and weight were dependent on chitosan concentration and molecular weight (Table 4). The maximum shoot and root length was obtained with the lowest molecular weight chitosan (2.27×10^5 g/mol) at the fivefold LC_{50} value whereas it was increased significantly (71.33 and 31.83 cm for shoot and root length, respectively) compared to the control (46.33 and 21.67 cm for shoot and root length, respectively). The fresh weight of tomato plants grown in untreated soil was 18.30 g/plant, whereas those from soils treated with Ch 1, Ch 2, Ch 3, and Ch 4 at the fivefold LC_{50} value were 48.67, 40.67, 38.43, and 34.57, respectively. However, no significant differences were observed in the fresh weight of tomato seedlings between the two treatments (one- and five-fold LC_{50} value). It can be noticed that Ch 1 was significantly the most active one among the other chitosans and oxamyl in the increasing of the total fresh weight of tomato seedlings with 46.10 and 48.67 g/plant was found at one- and five-fold treatments, respectively.

DISCUSSION

These results demonstrate the potential of the different molecular weights of a biopolymer chitosan to control the root-knot nematode, *M. incognita*. All trials resulted in good nematicidal activity against the tested nematode. Chitosan has useful nematistatic and nematicidal activity for agricultural and horticultural applications by admixing nematicidally effective amounts to a plant growth medium. The complex also provides a source of nitrogen in slow-release form, making it particularly suitable for combination with fertilisers and soil conditioners (Cardenas-Trivino *et al.* 1989; Vasyukova *et al.* 2001; Aboud *et al.* 2002; Kalaiarasan *et al.* 2006). Chitosan also reduced the extent of nematode invasion of plants and affected the morphophysiological and population parameters of *M. incognita*. Aboud *et al.* (2002) tested the chitosan against the infection of *M. javanica* in a greenhouse and field trial on tomato plants. Treatments comprised five chitosan rates and three application methods (soaking, root dipping, and spraying) alone or in combinations. They found that the most effective treatments were soaking tomato seeds or dipping the roots in a solution of 1.5 mg/ml for 20 minutes. Under field conditions, plants of soaked seeds with chitosan (1.5 mg/ml) showed a significant reduction in nematode infection parameters. However, the treatment with chitosan potentially induced systemic acquired resistance in tomato plants. Jayakumar *et al.* (2004)

used chitin waste materials such as crab shells, prawn wastes, and fish meal (each at 10 g/pot) against the root-knot nematode *M. incognita* infecting tomato plants under glasshouse conditions. Prawn shells showed a maximum decrease of up to 55.5% in the root-knot disease incidence compared to the control, followed by crab shells and fish meal. Our results are also in agreement with Vasyukova *et al.* (2001), who found that the low-molecular-weight chitosan (5 kDa) was shown to display an elicitor activity by inducing the local and systemic resistance of *L. esculentum* tomato to the root-knot nematode *M. incognita*. At all concentrations studied (100–1000 mg/ml), the chitosan caused elongation of the stem and accumulation of green biomass and all parameters of plant growth were higher than in the control. Chitosan at a concentration of 500 mg/ml was most potent in stimulating the growth of tomatoes. The number of knots on tomato roots decreased more than two-fold, the time of development of nematodes and their physiological state changed considerably, and the fertility of nematodes was 1.5-fold lower than that in the control.

The exact mechanisms of the nematicidal activity of chitosan and its derivatives are still unknown. However, it is known that the interaction between positively charged chitosan molecules (charge on the C-2 of the glucosamine monomer) and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents (Rabea *et al*. 2003; Badawy & Rabea 2011). The larger number of positive charges may have imparted a net positive charge to the surfaces to keep them in suspension as in microorganisms. Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth (Cuero *et al*. 1991). It also activates several defence processes in the host tissue (El Ghaouth *et al*. 1992), acts as a water-binding agent and inhibits various enzymes. Another mechanism is that the positively charged chitosan interacts with cellular DNA of some microorganisms, which consequently inhibits the RNA and protein synthesis; however, this mechanism is still controversial (Hadwiger *et al*. 1986).

In agriculture, chitosan has been used to increase the plant product, to stimulate the immunity of plants and to protect plants against microorganisms. A positive effect of chitosan was observed on the growth of roots, shoots, and leaves of several crop plants (Chibu & Shibayama 2001; Nge *et al*. 2006). The present study showed that chitosan compounds enhanced the tomato seedling growth to a different extent over the controls. This finding is in agreement with earlier observations made by many scientists who confirmed that chitosan enhanced the growth parameters of plant seedlings. For example, No *et al.* (2003) observed an increase in the weight of soybean sprouts from 6.9% to 29.2% after chitosan treatment. Lee *et al.* (1999) reported that the length of soybean (Korean cultivar Iksan) sprouts treated with chitosan was 25.4% longer than that of the control. Zinov'eva *et al.* (1999) reported that chitosan stimulated the growth and development of infected tomato plants by inducing plant resistance through increased lipoxygenase activity. The stimulatory effect was enhanced according to the increase of chitosan concentration (1, 10, and 100 µg/ml).

In conclusion, our results suggest that chitosans have the potential to be used for nematode control. Because these compounds and their derivatives, such as *N,O*-(acyl) chitosans, *N*-(benzyl) chitosans and quaternary *N*-alkyl chitosans, have also been reported to have fungicidal and antibacterial activities (Rabea *et al.* 2003, 2009; Badawy *et al.* 2004; Badawy 2010). Soil treatment with these compounds could serve as a soil disinfectant. However, further experiments are needed to evaluate economic aspects and nematicidal activity under field conditions with other nematode species and in other types of soil.

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