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Effect of chitosan on root-knot nematode, *Meloidogyne javanica* on tomato plants

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Abstract: In our study two different chitosan with low and high molecular weight at different dilutions i.e. standard, 1:1, 1:2, 1:3, 1:4, 1:5 and 1:10 were evaluated against root-knot nematode, *Meloidogyne javanica in vitro* and *in vivo* under greenhouse conditions. *In vitro* results revealed that both chitosan with all used concentrations significantly reduced the egg hatching when compared to control. No hatched eggs were shown with the standard concentration of both chitosan. The high molecular weight chitosan gave no significant results in egg hatching with the most evaluated concentrations i.e. standard, 1:1, 1:5 and 1:10 compared to the control. *In vitro* results revealed that both low and high molecular weight chitosan significantly affected the larvae mortality of *M. javanica* at all evaluated dilutions concentrations compared to control. Results found that standard, 1:1 and 1:2 dilutions were the most effective concentrations of the high molecular weight chitosan as they prevent completely the larvae to live. *In vivo* results revealed that both chitosan with all evaluated concentrations significantly reduced nematode parameters i.e. number of galls, females, developmental stages/root system and number of juveniles/250 g soil. Standard concentration of the high molecular weight chitosan was the highest effective one on nematode parameters as the reduction percentage was 92, 97, 92, 88 and 79%, respectively. The same trend of results was recorded with the antioxidant enzymes i.e. peroxidase and phenoloxidase as the standard concentration of both chitosan was encouraged the contents of enzymes compared to the treated plants with nematode alone. Results found also that both chitosan at all evaluated concentrations markedly enhanced the plant growth parameters i.e. root and shoot length and weights compared to the plants treated with nematode alone.
Key Words: Bio-control, *Meloidogyne* spp., Tomato, *Lycopersicon esculentum.*

Introduction

Root-knot nematodes, *Meloidogyne* spp. are considered the most damaging nematode group in the world as they cause high yield losses to most cultivated plant species in subtropical and tropical regions. Vegetable production in tropical and subtropical areas is highly dependent on suitable plant parasitic nematode control especially against *Meloidogyne* spp. which are usually the most damaging and tomato is one of the most vegetable crops attacked by these pests in the Miditerranean area[1,2]. Root-knot nematodes are among the most damaging nematodes in agriculture and consider one of the major limiting factors affecting plant growth and yield causing an estimated $100 billion loss/year worldwide[3].

Root-knot nematodes are also difficult to control due to their wide host range, short generation times, high reproductive rates and endoparasitic nature[4,5].

Synthetic pesticides, though instantaneously effective are usually prohibitively expensive, not readily

available, may cause hazards to both man and livestock and inflict injury to the environment[6]. During the last decades, nematologists worldwide search the cheaper, safer and eco-friendly alternatives methods i.e. biological and cultural methods to control the plant-parasitic nematodes.

Chitosan is one of the promising materials against plant pathogens as it exhibits a variety of antimicrobial activities and exhibits a hypersensitive response to infection[7]. Chitosan have demonstrated antiviral, antibacterial and antifungal properties and have been explored for many agricultural uses. They have been utilized to control disease or reduce their spread to chelate nutrient and minerals, preventing pathogens from accessing them or to enhance plant innate defenses[8]**.** Our research aimed to use high and low molecular weight chitosan to suppress and control root-knot nematode, *M. javanica* on tomato plants under laboratory and greenhouse conditions.

Materials and Methods

Pure culture of *M. javanica* was established from single egg masses on tomato plants under greenhouse conditions at 25±2°C. Nematode species identification was carried out according to the perineal patterns technique[9]. Root-knot nematode eggs were extracted from heavily galled roots by using 1.5% sodium hypochlorite solution (NaOCl) technique as described by[10]**.** Two thousand nematode eggs were pipetting into three holes made around tomato root zone at the same time of transplanting, except the treatment of 5% one week before nematode inoculation. Each treatment replicated three times and the non-treated plants were served as control. Plants were arranged in a completely randomized block design in the greenhouse at approximately 25±2°C. Plants were watered daily and fertilized weekly with 5 ml of 2 g/l N: P: K (20:20:20), International Egypt Company for Agricultural and Industrial Developing.

Egg masses were stained prior to counting by dipping the infected roots in phloxine-B solution (0.015%) for 20 minutes as described by[11]**.** Females were collected by cutting the root system of each plant in 2 cm pieces and submerging the roots in a beaker full of tap water for 4 day at room temperature until the root pieces became soft. The roots were then washed with tap water through 250 and 500 mesh sieves to separate the females from the root debris[12]. Larvae were extracted according to[13].

Preparation of high molecular weight chitosan

The production of chitosan involved the demineralization (DM), deproteinization (DP), decolorization (DC), and deacetylation (DA) steps[14]. Shrimp shell was demineralized with 1N HCl for 30 min at ambient temperature with a solid/solvent ratio of 1:15 (w/v). Following the DM step, the demineralized shell was collected on a 100-mesh sieve, washed to neutrality in running tap water, rinsed with deionized water, and filtered to remove excess moisture. The DP step was accomplished by treating the demineralized shell with 3% NaOH for 15min at 15 psi/121℃ and a solid/solvent ratio of 1:10 (w/v). The residue was then washed and filtered as mentioned above. For the DC step, the resulting chitin residue was bleached with 10% sodium hypochlorite solution for 5min with a solid/solvent ratio of 1:10 (w/v). The bleached chitin was collected, washed as mentioned above, and dried at 60℃ for 4 h in a forced-air oven or by sun drying (approximately at 23℃) for 4 hrs. The DA step was achieved by treating chitin under conditions of 15 psi/121 ℃ with 45% NaOH for 30min and a solid/solvent ratio of 1:10 (w/v). The resulting chitosan was collected, washed as mentioned above, and dried at 60℃ for 4 hrs. in a forced-air oven or by sun drying (approximately at 23℃) for 4 hrs.

Preparation of low molecular weight chitosan:

One gram of high molecular chitosan was added into 20 ml of 2% acetic acid (v/v) in a water-bath shaker (SHZ-82A, Henfeng Instrument Company, Jintan, China). The conditions were set as follows: H_2O_2 level (5.5%), time (3.5 h) and temperature (42.8 ℃). After reaction, 10% NaOH was used to adjust the solution to neutrality. The residue was removed by filtration, while two fold volumes of ethanol were added to the filtrate. The crystal of water-soluble chitosan was liberated after incubation at ambient condition overnight and dried in an air oven at 50℃[15].

Determination of molecular weight

The molecular weight of chitosan samples were determined by using an Ubbelohde viscometer at 30° C. the intrinsic viscosities (η) were determined, the solvent was 5% acetic acid and 0.1 M KCl the obtained

intrinsic viscosities were used to calculate molecular weight for the prepared samples from the Mark–Houwink–Sakurada relation: $\eta = KM^a$ where K and a are constants which K= $8.93x10^{-4}$ and a = 0.71 [16]. The high molecular weight chitosan was (50 Kda) and low molecular weight chitosan was (470 Kda).

Detremination of deacetylation degree of chitosan

The deacetylation degree of chitosan was determined by the potentiometric titration method described by Broussignac, as reported by [17]. Chitosan was dissolved in a known excess of hydrochloric acid. From the titration of this solution with a 0.1 M sodium hydroxide solution, a curve with two inflexion points was obtained. The difference between the volumes of these two inflexion points corresponded to the acid consumption for the salification of amine groups and permitted the determination of chitosan's acetylation degree, through Eq.

$\% NH_2 = 16.1 (V2 - V1) \times Mb / W$

Where (V1) and (V2) are the base volumes referred to first and second inflexion points, respectively, in ml, Mb is the base molarity in g/mol and W is the original weight of the polymer in g. The degree of deacetylation of chitosan was 80%.

Two months after nematode inoculation, nematode and growth parameters were recorded. The recorded nematode parameters were: numbers of galls, egg masses, females, developmental stages/root system as well as number of juveniles in soil pots[18].

Plant growth parameters i.e. shoot and root lengths and fresh weight were recorded as well as the antioxidant enzymes activity i.e. phenoloxidase and peroxidase in fresh leaves were also determined according to[19 , 20] .

Results

Results of *In vitro* test revealed that using both high molecular weight and low molecular weight chitosan at different concentrations significantly reduced egg hatching when compared to control (eggs in water). No hatched eggs were showed with standard (S) concentration of both HMW and LMW-chitosan (Fig. 1). The HMW-chitosan gave no significant results in egg hatching with the most evaluated concentrations i.e. S, 1:1, 1:3, 1:5 and 1:10 compared to control as shown in Fig.(1).

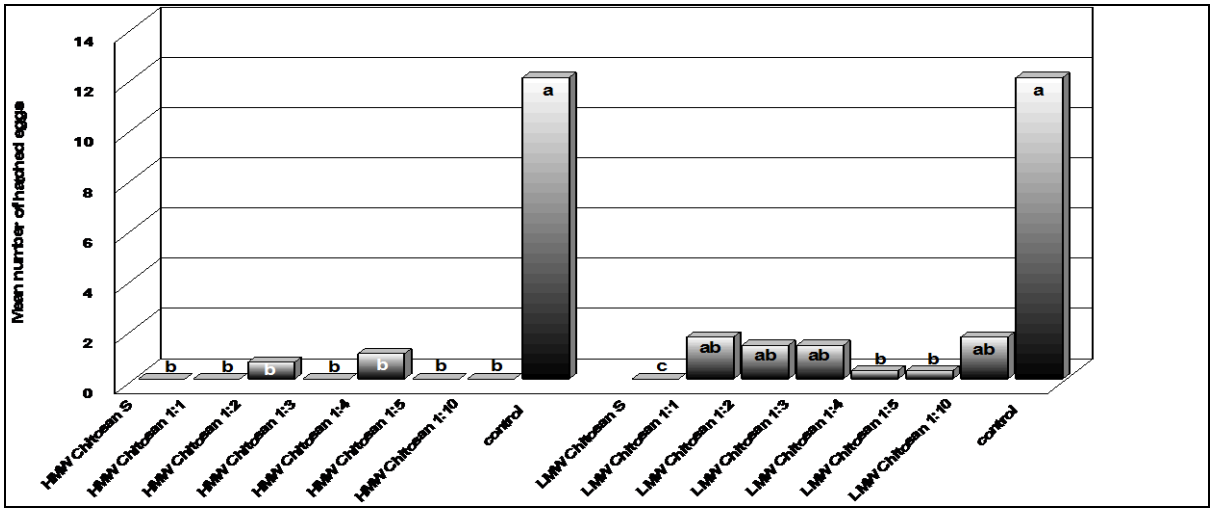

Figure (1): Effect of two different chitosan with high and low molecular weight on egg hatching of *M. javanica*.

Larvae mortality of *M. javanica* was significantly affected *In vitro* with both LMW and HMW-chitosan with all concentrations compared to control (larvae in water). Results found that the concentrations of S, 1:1 and 1:2 were the most effective treatment of HMW-chitosan as they prevent completely the larvae to live as shown in Fig. (2).

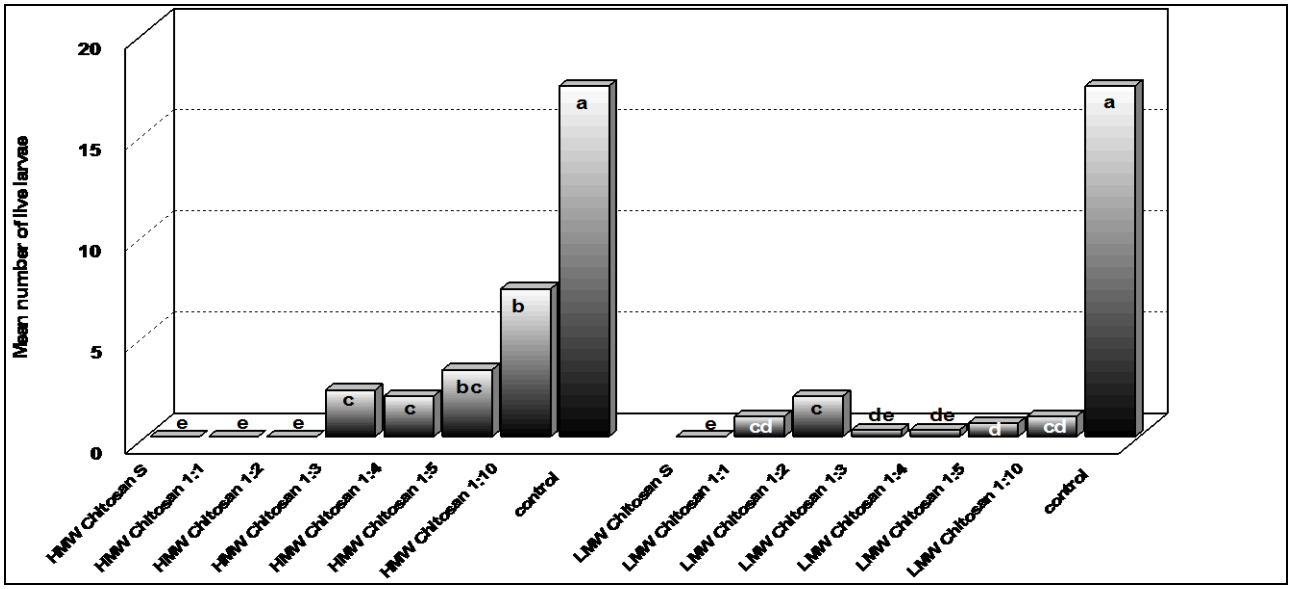

Figure (2): Effect of two different chitosan with high and low molecular weight on larvae mortality of *M. javanica*.

In vivo results revealed that both HMW and LMW-chitosan with all evaluated dilutions significantly reduced nematode parameters i.e. number of galls, egg masses, females/root system and number of juveniles/250 g soil. Standard concentrations of HMW and LMW-chitosan was the highest effective one on nematode parameters as the reduction percentage was 93, 97, 92 and 79%, and 92, 92, 77 and 92 %, respectively as shown in Table (1).

Concerning number of galls, egg masses and females/root system, results in Table (1) showed no significant differences between HMW and LMW-chitosan at all tested concentrations compared to plants treated with nematode alone. The exception showed with number of juveniles/250 g soil as the significant differences were showed between the different treatments and the treated plants with nematode alone.

Table (1): Effect of HMW and LMW-chitosan on controlling root-knot nematode, *M. javanica* on tomato plants.

Treatment	Dilutions	Galls / root system	Red. %	Egg masses / root system	Red. %	Juveniles / 250 g soil	Red. %	Females / root system	Red. %
HMW Chitosan	S (2.5%)	09.7 **b**	92	2.3 **b**	97	166.7 **e**	79	2.7 **b**	92
	1:1	11.3 **b**	93	2.7 **b**	96	283.3 **d**	64	2.7 **b**	92
	1:2	16.7 **b**	89	7.3 **b**	89	316.7 **cd**	60	5.7 **b**	84
	1:3	18.0 **b**	88	7.3 **b**	89	333.3 **cd**	57	7.3 **b**	79
	1:4	19.7 **b**	86	4.3 **b**	94	333.3 **cd**	57	3.3 **b**	90
	1:5	21.0 **b**	86	9.7 **b**	86	366.7 **bc**	53	4.3 **b**	88
	1:10	25.7 **b**	82	10.0 **b**	85	416.7 **b**	47	7.0 **b**	80
LMW Chitosan	S (2.5%)	12.3 **b**	92	5.7 **b**	92	183.3 **e**	77	2.7 **b**	92
	1:1	14.7 **b**	90	6.3 **b**	91	183.3 **e**	77	2.7 **b**	92
	1:2	24.3 **b**	83	9.0 **b**	87	200.0 **de**	74	5.3 **b**	84
	1:3	29.7 **b**	80	11.3 **b**	83	316.7 **cd**	60	7.7 **b**	78
	1:4	33.3 **b**	77	12.0 **b**	82	333.3 **cd**	57	9.7 **b**	72
	1:5	29.0 **b**	80	11.3 **b**	83	450.0 **b**	43	7.7 **b**	78
	1:10	33.7 **b**	77	11.7 **b**	83	466.7 **b**	40	11.0 **b**	68
Nematode alone		**145.3 a**	-	**68.3 a**	-	**783.3 a**	-	**34.7 a**	-

HMW= high molecular weight **LMW= low molecular weight**

Table (2): Effect of HMW and LMW- chitosan on controlling root-knot nematode, *M. javanica* on tomato plants.

Treatment	Dilutions	Shoot Fresh weight (g)	Eff. %	Root Fresh weight (g)	Eff. %	Shoot Length (cm)	Eff. %	Root Length (cm)	Eff. %
HMW Chitosan	S (2.5%)	8.5 **a**	750	2.9 **a**	190	15.8 **a**	690	6.6 **a**	560
	1:1	2.6 **cd**	160	1.9 **b**	90	7.2 **bc**	260	3.2 **b**	220
	1:2	3.7 **c**	270	0.7 **c**	-	8.2 **b**	310	2.7 **bc**	170
	1:3	6.2 **ab**	520	1.5 **bc**	50	10.1 **ab**	405	4.7 **ab**	370
	1:4	5.8 **b**	480	1.3 **bc**	30	2.5 **d**	50	3.2 **b**	220
	1:5	1.9 **d**	90	2.0 **ab**	100	6.1 **bc**	205	1.0 **c**	-
	1:10	2.3 **cd**	130	1.9 **b**	90	3.5 **c**	75	2.8 **bc**	180
LMW Chitosan	S (2.5%)	8.0 **a**	700	2.1 **ab**	110	10.7 **ab**	435	6.4 **a**	540
	1:1	3.6 **c**	260	1.4 **bc**	40	8.2 **b**	310	1.0 **c**	-
	1:2	3.8 **c**	280	1.1 **c**	10	8.7 **b**	335	3.1 **b**	210
	1:3	3.3 **c**	230	0.6 **c**	-	8.0 **b**	300	3.8 **b**	280
	1:4	3.2 **c**	220	1.0 **c**	-	4.2 **c**	110	1.0 **c**	-
	1:5	2.2 **cd**	120	0.7 **c**	-	3.8 **c**	90	4.4 **ab**	330
	1:10	1.5 **d**	50	0.4 **c**	-	2.5 **d**	50	2.1 **bc**	110
Nematode alone		1.0 **d**	-	1.0 **c**	-	2.0 **d**	-	1.0 **c**	-

HMW= high molecular weight **LMW= low molecular weight**

Efficacy % = Treatment - N alone / N alone X 100.

Results in Table (2) revealed that plant growth parameters i.e. shoot and root fresh weight (g) and length (cm) were significantly affected by application both HMW and LMW-chitosan at all tested dilutions when compared to plants treated with nematode alone.

Standard concentrations of both HMW and LMW-chitosan were the highly effective one in enhancing all growth parameters as the efficacy% recorded the highest values compared to the other treatments (Table, 2). Standard concentration of HMW –chitosan gave 750, 190, 690 and 560% enhancement shoot and root fresh weight and length, respectively, whereas LMW-chitosan gave 700, 110, 435 and 540%, respectively. The lowest values of efficacy were recorded with the low concentration 1:10 of LMW-chitosan by 50, 50 and 110% of fresh shoot weight, shoot and root length, respectively compared to nematode alone (Table, 2).

Table (3): Effect of HMW and LMW-chitosan on controlling root-knot nematode, *M. javanica* on tomato plants.

Treatment	Dilutions	Peroxidase	Phenoloxidase
HMW chitosan	S (2.5%)	1.00 **a**	1.50 **a**
	1:1	0.90 **b**	0.90 **c**
	1:2	1.00 **a**	0.80 **d**
	1:3	0.70 **c**	1.00 **b**
	1:4	0.60 **d**	1.00 **b**
	1:5	0.90 **b**	0.70 **ef**
	1:10	0.50 **e**	0.62 **fg**
LMW chitosan	S (2.5%)	1.00 **a**	1.40 **a**
	1:1	0.62 **d**	0.70 **ef**
	1:2	0.60 **d**	0.70 **ef**
	1:3	0.90 **b**	0.52 **g**
	1:4	0.60 **d**	0.60 **fg**

	1:5	0.62 **d**	0.52 **g**
	1:10	0.42 **f**	0.50 **g**
Nematode alone		0.30 **g**	0.40 **h**
Control		0.50 **e**	0.60 **fg**

The same trend of results was recorded with the antioxidant enzymes i.e. peroxidase and phenoloxidase as both chitosan types with all tested dilutions enhanced the activity of both enzymes compared to the plants treated with nematode alone as shown in Table (3). The standard concentration of both chitosan were significantly encouraged the contents of enzymes compared to the treated plants with nematode alone (Table, 3). Both chitosan at the dilution 1:10 showed the lowest effect between the other concentrations on enzymes activity of peroxidase and phenoloxidase.

Discussion

Results of *in vitro* tests revealed that both chitosan with all evaluated concentrations significantly reduced the egg hatching and enhanced the larvae mortality of *M. javanica*.

Results of *in vivo* tests found also that both HMW and LMW-chitosan at all concentrations significantly reduced all nematode parameters compared to plants treated with nematode alone.

Cchitosan used to control plant pathogens has been extensively explored with more or less success depending on the pathosystem, the used derivatives, concentration, degree of deacylation, viscosity, and the applied formulation (i.e., soil amendment, foliar application; chitosan alone or in association with other treatments) [21].

Chitosan is naturally-occurring compound that have potential in agriculture with regard to controlling plant diseases. This molecule was shown to display toxicity and inhibit fungal growth and development. They were reported to be active against viruses, bacteria and other pests as reported by [22]**.** They found also that chitosan is known to have eliciting activities leading to a variety of defense responses in host plants in response to microbial infections, including the accumulation of phytoalexins, pathogen-related (PR) proteins and proteinase inhibitors, lignin synthesis, and callose formation.

Chitosan exhibits a variety of antimicrobial [23,24,25]**,** which depend on the type of chitosan (native or modified), its degree of polymerization, the host, the chemical and/or nutrient composition of the substrates, and environmental conditions.

Chitosan utilized as a soil amendment was shown to control Fusarium wilts in many plant species [26]**.**

The other part is linked to that this biopolymer is composed of polysaccharides that stimulate the activity of beneficial microorganisms in the soil such as Bacillus, fluorescent Pseudomonas, actinomycetes, mycorrhiza and rhizobacteria [27,28]**.**

Chitosan is often used in plant disease control as a powerful elicitor rather than a direct antimicrobial or toxic agent. Its direct toxicity remains dependent on properties such as the concentration applied, the molecular weight, degree of acetylation, solvent, pH and viscosity [29,30].

Tthe effect of chitosan in date palm in response to *Fusarium oxysporum f. sp. albedinis*, the causal agent of a major wilt in this crop. Beside a direct toxicity of the molecule on the fungus, the authors showed an enhancement of essential components of the host resistance. When injected into the roots at various concentrations, chitosan elicited date palm peroxidase and polyphenoloxidase activities, and increased the level of phenolic compounds. Among the accumulated phenolics, there was an increase in content of specific non-constitutive hydroxycinnamic acid derivatives, known to be of great importance in the resistance of this plant to this vascular fusariosis [31].

Chitosan, when applied to plant tissues, often agglutinate around the penetration sites and has two major effects. The first one is the isolation of the penetration site through the formation of a physical barrier preventing the pathogen from spreading and invading other healthy tissues. This phenomenon resembles the

abscission zones often observed on leaves preventing several necrotrophic pathogens from spreading further. It is widely observed on potato tubers for example [32]. Around the isolated zones, often an elicitation of a hypersensitive response occur with the accumulation of H_2O_2 that helps in cells wall fortification and serve as an alert signal for other healthy parts of the plant. The second effect is due to the chitosan' ability to bind various materials and initiate fast the wound healing process [33].

Chitosans are well used in the fresh and salt water purification process as chelators for minerals and metals. These abilities are also explored when chitosan is applied to plants to prevent diseases because it can chelate nutrients and minerals (*i.e.*, Fe, Cu), preventing pathogens from accessing them. These polysaccharide molecules were also reported to bind mycotoxins [34], which may reduce damage to the host tissues due to toxins. In the beverage industry, for example, chitosan and derivatives are often used for their antimicrobial properties linked to their chelating abilities of nutrient and minerals, thus reducing fungal spoilage [35].

References

1. Molinari, S. and Baser, N. (2010). Induction of resistance to root-knot nematodes by SAR elicitors in tomato. Crop Protection 29(11): 1354-1362.
2. Sikora, R. A. and Fernàndez, E. (2005). Nematode parasites of vegetables. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, UK, pp. 319-392.
3. Oka, Y.; Spiegel, Y. and Cohen, Y. (2000). β–amino-butyric acid-induced resistance in plants against nematodes. Phytoparasitica 28(3): 274.
4. Manzanilla-Lopez, R. H.; Kenneth, E. and Bridge, J. (2004). Plant diseases caused by nematodes. In: Chen, Z.X., Chen, S.Y., Dickson, D.W. (Eds.), Nematology Advances and Perspectives. Nematode Management and Utilization, vol. II. CAB International, Wallingford, UK, pp. 637-716.
5. Trudgill, D. L. and Blok, V. C. (2001). Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Annual Review of Phytopathology 39: 53-77.
6. Mukhtar, T.; Hussain, M. A.; Kayani, M. Z. and Aslam, M. N. (2014). Evaluation of resistance to root-knot nematode (*Meloidogyne incognita*) in okra. Crop Protection 56: 25-30.
7. Kulikov, S. N.; Chirkov, S. N.; Il'ina, A. V.; Lopatin, S. A. and Varlamov, V. P. (2006). Effect of the molecular weight of chitosan on its antiviral activity in plants. Prik. Biokhim. Mikrobiol. 42 (2): 224–228.
8. El-Hadrami, A.; Lorne R. A.; El-Hadrami, I. and Daayf, F. (2010): Chitosan in Plant Protection. Mar. Drugs 8: 968-987.
9. Taylor, A. L. and Sasser, J. N. (1978). Biology, identification and control of root-knot nematodes, Meloidogyne spp. International Meloidogyne Project Publication, North Carolina State Graphics Univ., Raleigh, p. 111.
10. Hussey, R. S. and Barker, K. R. (1973). A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57: 1025-28.
11. Daykin, M. E. and Hussey, R. S. (1985). Staining and histopathological techniques in nematology. In: Barker, K. R., C. C. Carter and J. N. Sasser (eds), An advanced treatise on Meloidogyne, Vol. II. Methodology, pp. 39-48. North Carolina State University Graphics, Raleigh.
12. Mahdy, M. E. (2002). Biological control of plant parasitic nematodes with antagonistic bacteria on different host plants. Ph.D. Thesis, Bonn University, Germany, pp.171.
13. Oostenbrink, M. (1960). Estimating nematode population by some selected methods. Pp.85-102 in: Sasser, J. N. and Jenkins, W. R. (eds) . Nematology Raleigh, N.C:North Carolina State University Press, USA.
14. No, H. K.; Lee, S. H.; Park, N. Y. and Meyers, S. P. (2003). Comparison of physicochemical, binding, and antibacterial properties of chitosans prepared without and with deproteinization process. Journal of Agricultural and Food Chemistry 51: 7659 - 7663.
15. Yunjian Du; Yuqiao Zhao; Shuchao Dai and Bao Yang (2009). Preparation of water soluble chitosan from shrimp shell and its antibacterial activity. Innovative Food Science and Emerging Technologies 10: 103–107.
16. Chandumpaia, A.; Singhpibulpornb, N.; Faroongsarngc, D. and Sornprasit, P (2004). Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, *Loligo lessoniana* and *Loligo formosana*. Carbohydrate Polymers 58: 467–474.

17. Tolaimate, A.; Desbrie`res, J.; Rhazi, M.; Alagui, A.; Vincendon, M. and Vottero, P. (2000). On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. *Poly*. 41: 2463–2469.
18. Goodey, J. B. (1957). Laboratory methods for work with plant and soil nematodes. Tech. Bull. No.2, Min. Agric. Fish, Ed. London, pp.47.
19. Broesh, S. (1954). Colorimetric assay of phenoloxidase. Bull. Sci. Chem. Biol., 36: 711-713.
20. Fehrman, H. and Dimond, A. E. (1967). Peroxidase activity and *Phytophthora* resistance in different ranges of potato. Plant Pathology 57: 69-72.
21. El-Hadrami, A.; Lorne R. A.; El-Hadrami, I. and Daayf, F. (2010): Chitosan in Plant Protection. Mar. Drugs 8: 968-987.
22. El-Hadrami, A.; Lorne R. A.; El-Hadrami, I. and Daayf, F. (2010): Chitosan in Plant Protection. Mar. Drugs 8: 968-987.
23. Pospieszny, H.; Chirkov, S. and Atabekov, J. (1991). Induction of antiviral resistance in plants by chitosan. Plant Sci. 1991, *79*, 63–68.
24. Rabea, E. I.; El Badawy, M. T.; Stevens, C. V.; Smagghe, G. and Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. Biomacromolecules, 4: 1457–1465.
25. Kulikov, S. N.; Chirkov, S. N.; Il'ina, A. V.; Lopatin, S. A. and Varlamov, V. P. (2006). Effect of the molecular weight of chitosan on its antiviral activity in plants. Prik. Biokhim. Mikrobiol. 42 (2): 224–228.
26. Rabea, E. I.; El Badawy, M. T.; Stevens, C. V.; Smagghe, G. and Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. Biomacromolecules, 4: 1457–1465.
27. Bell, A. A.; Hubbard, J. C.; Liu, L.; Davis, R. M. and Subbarao, K. V. (1998). Effects of chitin and chitosan on the incidence and severity of Fusarium yellows in celery. Plant Dis., 82, 322–328.
28. Murphy, J. G.; Rafferty, S. M. and Cassells, A. C. (2000). Stimulation of wild strawberry (*Fragaria vesca*) arbuscular mycorrhizas by addition of shell fish waste to the growth substrate: interaction between mycorrhization, substrate amendment and susceptibility to red core *(Phytophthora fragariae*). Appl. Soil Ecol., 15: 153–158.
29. No, H. K.; Young, P. N.; Ho, L. S.; Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. Food Microbiol., 74: 65–72.
30. Chung, Y. C.; Wang, H. L.; Chen, Y. M. and Li, S. L. (2003). Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. Bioresour. Technol., 88: 179 –184.
31. El Hassni, M.; El Hadrami, A.; Daayf, F.; Chérif, M.; Ait Barka, E. and El Hadrami, I. (2004). Chitosan, antifungal product against *Fusarium oxysporum* f. sp. *albedinis* and elicitor of defence reactions in date palm roots. Phytopathol. Mediterr., 43: 195–204.
32. El Hadrami, A.; El Hadrami, I. and Daayf, F. (2009). Suppression of induced plant defense responses by fungal pathogens. In *Molecular-Plant Microbe Interactions*; Bouarab, K., Brisson, N., Daayf, F.,Eds.; CABI: Wallingford, UK,; Chapter 10, pp. 231–268.
33. Hirano, S.; Nakahira, T.; Nakagawa, M. and Kim, S. K. (1999). The preparation and applications of functional fibers from crab shell chitin. *J. Biotechnol.*, *70*: 373–377.
34. Bornet, A. and Teissedre, P. L. (2007). Chitosan, chitin-glucan and chitin effects on minerals (iron, lead, cadmium) and organic ochratoxin A) contaminants in wines. *Eur. Food Res. Technol.*, *226* (4), 681–689.
35. Tsai, G. J. and Su, W. H. (1999). Antibacterial activity of shrimp chitosan against *Escherichia coli*. *J. Food Prot.*, *62*: 239–243.

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