International Journal of Agriculture, Environment and Food Sciences
e-ISSN : 2618-5946
www.jaefs.com
JAEFS
edit PUBLISHING

Research Article
Int J Agric Environ Food Sci 6 (3): 410-416 (2022)

Nematicidal effect of chitosan on *Meloidogyne incognita in vitro* and on tomato in a pot experiment

Fatma Gül Göze Özdemir[1,*] Hacer Çevik[1] Jean Claude Ndayiragije[1]
Tuğçe Özek[1] İsmail Karaca[1]**

[1]Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection, Isparta, Turkey

[*]Corresponding Author: fatmagoze@isparta.edu.tr
[**]100/2000 YÖK Doktora Bursiyeri

Citation
Goze Ozdemir, F.G., Cevik, H., Ndayiragije, J.C., Ozek, T., Karaca, I. (2022). Nematicidal effect of chitosan on *Meloidogyne incognita in vitro* and on tomato in a pot experiment. International Journal of Agriculture, Environment and Food Sciences, 6 (3), 410-416.
Doi: https://doi.org/10.31015/jaefs.2022.3.10

Received: 05 July 2022
Accepted: 21 July 2022
Published Online: 09 August 2022

Year: 2022
Volume: 6
Issue: 3 (September)
Pages: 410-416

Available online
http://www.jaefs.com
https://dergipark.org.tr/jaefs

Abstract
The present study investigated to evaluate the potential of liquid chitosan of three concentrations (0.5, 1 and 2%) on *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 *in vitro* and on tomato under controlled conditions ((24 ± 1 °C, 60 ± 5% humidity). *In vitro* assay, the effect of the liquid chitosan concentrations on suppressing of hatching from eggs and second juvenile larvae (J2) mortality was determined. The reducing effect of the concentrations on the number of gall and egg mass on tomato roots and the J2 density in the soil was evaluated under controlled conditions. All concentrations suppressed hatch and increased J2 mortality more than control *in vitro*. The most effective concentration was found at 2% *in vitro* and its nematicidal effect on egg and J2 was over 70%. The results demonstrated that 0.5, 1 and 2% concentrations were significantly decreased gall/root, egg mass/root and J2 in soil compared to negative control under controlled conditions. No statistically significant difference was found between the nematicidal effects of the concentrations on the gall and egg mass ($P \leq 0.05$). It has been determined that 1 and 2% concentrations better suppress the J2 in soil than 0.5%. Although the nematicidal effect of 2% concentration was high *in vitro* and under controlled conditions, it was determined that it negatively affected plant biomass. Also, only 1% concentration of chitosan application controlled *M. incognita* on tomato by 58%. The present results show that the use of 1% liquid chitosan concentration against *M. incognita* will be more effective.

Keywords
Biological Control, Chitosan, Nematicidal Effect, Root Knot Nematode

Introduction
The most common root-knot nematode species worldwide are *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, and *Meloidogyne hapla* Chitwood, 1949 (Collange et al. 2011; Seid et al. 2015). Within the studies conducted in Turkey, *M. incognita*, *M. javanica*, *M.arenaria*, *M. hapla*, *Meloidogyne chitwoodi* Golden, O'Bannon, Santo, and Finley, 1980, *Meloidogyne artiellia* Franklin, 1961, *Meloidogyne acrita* Chitwood, 1949, *Meloidogyne luci* Carneiro et al., 2014, *Meloidogyne exiqua* Goeldi, 1887 and *Meloidogyne thamesi* (Chitwood in Chitwood, Specht and Havis, 1952) species have identified (Aydınlı et al. 2013; Imren et al. 2014; Çetintaş and Çakmak 2016; Uysal et al. 2017; Aydınlı 2018; Gürkan et al. 2019; Arslan and Elekçioğlu 2022). The damages of these nematodes in the roots prevent the plant from taking nutrients and water from the soil; and this results in plant's yellowing, wilting and stunting. In addition, root knot nematodes weaken the defenses of the host plant, making the plant more susceptible to attacks by other plant pathogens (Goverse and Smant, 2014). Root-knot nematodes are difficult to control because of their wide host range, short reproduction times, high reproduction rates, endoparasitism nature and further formation of disease complexes with soil pathogens (Saad et al., 2022; El-Ashry et al., 2022). Control of this pest is mainly attained

with chemical nematicides. However, nematicide products account for only 2.5% of global pesticides. Considering the big losses from plant parasitic nematodes, this rate is actually low (Castaneda-Alvarez and Aballay, 2016). Also, many chemical nematicides have been banned or restricted due to safety or environmental concerns (Fan et al., 2022). Recently, studies on biocontrol agents based on biomolecules themselves or their derivatives have increased. These agents, which are eminent to pose less risks to humans and animals than their synthetic precursors, also have a selective mode of action, and prevent the development of pest species's resistances to specified active ingredients (Ntalli and Caboni, 2012). Chitin and chitosan macromolecules also come as alternative biological agents that can be used in pest control (Fan et al., 2022). So far, chitin and chitosan products are marketed as alternatives to synthetic nematicides for plant parasitic nematodes (Agrinos, 2014). Chitosan is commercially available and can be used in many forms, and so it seems more appealing than chitin (Stevens, 2005; Shahidi et al., 2005; Imamoğlu, 2011).

Chitosan (D-glucosamine) is a soluble polymer obtained from the deacetylation process of chitin (Struszczyk, 2001; Berger et al., 2011). Chitosan is biodegradable, safe and non-toxic. It has a significant ability to adhere tightly to mammalian and microbial cells. Chitosan can be used as a plant coating material, and this is among the reasons why it is preferred in various agricultural activities (El Hadrami et al., 2010). Chitosan has been reported to exhibit insecticidal, antiviral, antibacterial and antifungal properties (Rabea et al., 2005; Li et al., 2009; El Hadrami et al., 2010; Hashem et al., 2010; Goy et al., 2016; Jaber et al., 2021). While the effect of chitosan varies depending on its chemical structure, molecular weight, concentration, plant species and application conditions, it was reported to activate numerous biological responses in the plant, such as resistance to stress and increased yield (Malerba and Cerana, 2015; Kurtuluş and Vardar, 2020). Mouniga et al. (2022) revealed that 1% chitosan nanoparticles increased their phenol content, peroxidase and polyphenol oxidase activity, protecting plants from root knot nematode infection and creating systemic resistance against nematodes. Chitosan releases different toxic chemical compounds with lethal effects against the J2 period and reproduction of *M. incognita* during decomposition (Ashif et al., 2017). El-Sayed and Mahdi (2015) found that high molecular weight chitosan undiluted concentration nematicidal effect above 80% on *M. javanica*. It was found that when 100 and 200 g of chitin were added to 150 cm^3 soil two weeks before *M. incognita* inoculation, egg number of root knot nematode and galling in the roots have significantly reduced (Ladner et al., 2008).

In Turkey, there has been no study on the use of chitosan in nematode control. In the present study, the nematicidal effect of liquid chitosan polymer on *M. incognita* was evaluated *in vitro* and on tomato under controlled conditions.

Materials and Methods

Material

The study was carried out with liquid chitosan polymer and DR17 *M. incognita* isolate. Chitosan was obtained from Kıtosan A.Ş; Antalya, Turkey. The root knot nematode material used in the study was collected from the eggplant greenhouse of Deregumu in Isparta province, Turkey and was defined morphologically and molecularly by Uysal et al. (2017) in a previous study. Since root-knot nematodes are obligate, mass production is carried out on live plants and renewed every 2-3 months in Tueza F1 tomato variety under climate room conditions (24±1°C, 60%±5 humidity) in Isparta University of Applied Sciences (ISUBÜ), Faculty of Agriculture, Department of Plant Protection. Distilled water was used as negative control and 10 µg/ml Velum (Fluopyram, Bayer Grup Ltd. Şti) was used as positive control.

Methods

Preparation of Nematode Inoculum of *Meloidogyne incognita*

Egg masses were handpicked from galls of tomato roots. Then, the surface was sterilized with 0.5% sodium hypochlorite for 3 min and washed with sterile water 3 times. Egg masses were incubated in distilled water for 5 days at 28°C (Misiha et al., 2013). Hatched juveniles (J2) were collected daily using a micropipette and stored at 4°C. Eggs were extracted by centrifugating a suspension made of 0.5–1 cm long chopped infested tomato roots and 1% sodium hypochlorite for 5 min (Coolen and D'Herde, 1972). Eggs were poured on a 75 µm pore sieve and collected on 5 µm pore sieve then washed with tap water to remove sodium hypochlorite (Nico et al., 2004; Liu et al., 2008).

Determination of *In vitro* Nematicidal Effect

In the study, the nematicidal effects of 0.5%, 1% and 2% concentrations of liquid chitosan polymer on the egg mass, egg and J2 of *M. incognita* were evaluated. The study was carried out in a randomized plot design with 5 replications in autoclaved petri dishes, each with a diameter of 6 cm. After applications in all experiments were completed, Petri dishes were incubated at 25°C.

Effect on hatching from J2 in Egg Mass and Egg

In order to determine the nematicidal effect on the egg mass, two *M. incognita* egg masses of almost equal sizes were transferred with forceps to petri dishes containing 2 ml liquid chitosan suspension concentration. Healthy J2 individuals hatched from eggs were counted after 7 days and the rates of hatching suppression were calculated. To determine the effects to egg hatching, an amount of one ml of egg suspension (approximately 100 eggs) was poured into a petri dish, and then 2 ml of chitosan suspension according to the concentration was added to every petri dish. The hatched J2s were counted after 7 days and hatching suppression rates were calculated (Liu et al., 2008). The suppression rate of *M. incognita* suspended in chitosan liquid was calculated with the following formula. Suppression Rate = [(Application-Negative Control)/Negative Control) X100] (Karabörklü et al. 2022).

Effect on J2

After one ml of J2 suspension (approximately 100 J2) was poured into each petri dish, 2 ml of suspension was added to these petri dishes according to the concentration. Dead J2s were counted under the light microscope after 24 hours. Subsequently, then suppression rate on J2 was determined. The suppression rate of *M. incognita* suspended in chitosan liquid was calculated with the following formula. Suppression Rate = [(Application-Negative Control)/Negative Control) X100] (Karabörklü et al. 2022).

Chitosan Concentration Response Tests on *Meloidogyne incognita* Infestation on Tomato Roots

The study was carried out with 0.5%, 1% and 2% concentrations of liquid chitosan suspension under controlled conditions (24 ± 1 °C, 60 ± 5% humidity) and was set up in a randomized block design with 5 replications. The study was carried out on a three weeks old Alberty F1 tomato cultivar. Tomato seedlings were each transplanted into a plastic pot with a diameter of 6 cm containing approximately 300 g of sterile soil (68% sand, 21% silt and 11% clay). The next day, 2000 *M. incognita* eggs suspended into distilled water were evenly distributed in three holes drilled around each seedling (Elkelany et al., 2020). Immediately after nematode inoculation, 5 ml of each concentration of the liquid chitosan suspension was applied to each potting soil (Elkelany et al., 2020).

The treatment was terminated 60 days after application. After the data about plant height and wet weight were recorded, the roots were gently uprooted from the soil and were washed under tap water. Afterwards, wet root's biomass and lengths were recorded. Then, the number of galls and egg masses in the roots were observed under the stereo microscope. In addition, the *M. incognita* J2 density in 100 g soil was obtained using Baermann funnel method (Barker, 1985); and counted under the light microscope (with x40 magnification). The control rate on gall, egg mass and J2 density in soil were calculated with the following formula. Effectivity Rate= [(Negative control– Liquid chitosan concentration application)/Negative control] X100 (Karabörklü et al. 2022).

Statistical analyses

SPSS Version 20 (IBM Corporation, Armonk, New York, USA) program was applied for the statistical analysis. The values were shown as mean±standard deviation for results. All data were checked for the normality by using the Kolmogorov Smirnov and Shapiro-Wilk tests. Data conforming to normal distribution, one-way ANOVA and TUKEY test for multiple comparison was performed ($P \leq 0.05$).

Results and Discussion

***In vitro* Nematicidal Effect**

The rates of hatching suppression of J2 and mortality of J2 in 0.5, 1 and 2% concentrations of liquid chitosan were found to be significantly higher than the rates in the negative control ($P \leq 0.05$). The highest nematicidal effect was detected at 2% concentration. The suppressive effects of 2% liquid chitosan on the egg hatching of J2 and directly on J2 were determined within the same statistical group with the chemical nematicide, Velum. However, the percentage rate of hatching suppression in J2 from individual egg was found to be lower in 2% concentration (73.9%) than in Velum (95.6%). In 2% concentration, in vitro nematicidal effect of egg, egg masses and J2 were determined to be over 70% and there was no statistical difference between them ($P \geq 0.05$). While the nematicidal effect of 0.5% concentration on egg and egg mass was found to be above 50%, its suppressive effect on J2 was found to be below 20%. Nematicidal effect on J2 was found to be lower than the effect on eggs and egg masses at 1% concentration (Table 1).

Table 1. In vitro nematicidal effect of liquid chitosan concentration on *Meloidogyne incognita*

Treatment	Suppression rate of egg hatching (%)	Suppression rate of egg masses hatching (%)	Mortality rate of J2 (%)
	Mean±Standart error		
0.5%	50,1±0,8 d A*	61,2±1,2 b A	19,0±3,2 c B
1%	62,2±1,3 c A	66,1±1,5 b A	46,5±2,5 b B
2%	73,9±1,4 b A	78,6±0,6 a A	74,0±2,6 a A
Velum (Positive control)	95,6±2,3 a A	82,3±1,6 a A	83,6±5,4 a A
Distilled water (Negative control)	1,1±0,5 e	0,2±0,1 c	0,5±0,2 d

*The lowercase letters in the same column indicate significant differences between the means of treatments and different uppercase letters in the same row indicate the suppressive effect of the treatment on eggs and larvae ($P \leq 0.05$).

Chitosan Concentration Response Tests on *Meloidogyne incognita* Infestation on Tomato Roots

The lowest number of gall (9.4±2.0) and egg masses (11.0±2.4) on tomato roots and the J2 density in the soil (278.0±65.7) were determined in Velum (Fluopyram) treatment, whereas the highest gall (168.4±5.7), egg mass (173.0±5.6) and soil density (2262.0±109.0) were found in distilled water treatment, which is the negative control. In 0.5, 1 and 2% concentration trials, the number of gall and egg masses in the roots and J2 density in the soils were significantly decreased compared to negative control ($P \leq 0.05$). However, the nematicidal effect of 0.5, 1 and 2% concentrations on the roots were lower than the resulted effect in Velum. When the number of gall and egg mass were evaluated, there was no statistical difference between 0.5, 1 and 2% concentrations ($P \geq 0.05$). The soil density resulted as significantly higher at 0.5% concentration (1493.6±58.7) than at 1% (916.0±63.0) and 2% (956.8±42.1) ($P \leq 0.05$). Although the control effects of 0.5% concentration treatment of liquid chitosan on root gall and egg mass was lower than 1 and 2% concentration treatments, the difference between them was not statistically significant ($P \geq 0.05$). The control effect of chitosan treatment on tomato roots on gall and egg mass were found to be over 45%. Also, the control effect on the J2 soil density was found to be 33.9%, 59.4% and 57.6% at 0.5, 1 and 2% concentration treatments, respectively (Table 2).

Plant heights, plant biomass and root lengths had the highest values in nematode-free control, positive control (Velum) and 1% liquid chitosan treatments, and no statistical difference was detected between them ($P \geq 0.05$). Wet root weight was found to be lower in the positive control than the nematode-free control and 1% liquid chitosan treatment, and a statistical difference was determined between them ($P \leq 0.05$). Generally, there was no significant difference between 0.5 and 2% treatments of liquid chitosan and negative control (distilled water) in

terms of plant growth parameters. Plant growth parameters of 2% concentration of liquid chitosan were determined lower than that of 1%, and it was found that the plant was adversely affected at higher concentrations (Table 3).

Table 2. Nematicidal effect of liquid chitosan concentration on tomato roots infested with *Meloidogyne incognita*

Treatment	Number of gall/root	Control effect of gall	Number of egg masses/root	Control effect of egg masses	Number of J2 /100 g soil	Control effect of J2 in soil
			Mean ± Standart error			
0.5%	86,0±6,8 b*	49,1±4,0 b	89,6±7,7 b	48,1±4,4 b	1493,6±58,7 b	33,9±2,6 c
1%	69,6±8,5 b	58,7±5,0 b	72,0±8,6 b	58,3±4,9 b	916,0±63,0 c	59,4±2,8 b
2%	70,2±7,9 b	58,4±4,6 b	73,8±7,7 b	57,2±4,4 b	956,8±42,1 c	57,6±1,8 b
Velum (Positive control)	9,4±2,0 c	94,3±1,2 a	11,0±2,4 c	93,5±1,3 a	278,0±65,7 d	87,6±2,9 a
Distilled water (Negative control)	168,4±5,7 a		173,0±5,6 a		2262,0±109,0 a	

*Different letters in the same column indicate the significant differences between means (P≤0.05).

Table 3. Plant growth in tomato roots infected with *Meloidogyne incognita* in which liquid chitosan concentrations were applied

Treatment	Plant height (cm)	Plant wet weight (g)	Root height (cm)	Root wet weight (g)
		Mean ± Standart error		
%0.5	23,1±1,3 b*	6,4±0,2 b	14,5±1,7 ab	8,0±1,7 bc
%1	33,2±2,6 a	13,7±0,5 a	18,3±0,7 a	11,3±1,0 a
%2	16,6±2,0 b	4,7±0,8 b	10,0±1,1 b	4,9±1,4 c
Velum (Positive control)	37,2±0,7 a	13,6±0,8 a	15,6±1,4 ab	10,9±0,9 b
Distilled water (Negative control)	20,6±0,9 b	5,2±0,2 b	10,8±0,6 b	10,0±0,4 b
Nematode-free control	40,6±1,7 a	14,6±1,1 a	17,4±2,3 a	11,3±0,8 a

*Different letters in the same column indicate the significant differences between means (P≤0.05).

In vitro study showed that chitosan treatments significantly reduced the hatching of J2 from eggs and increased the mortality of J2. It was determined that the nematicidal effect on eggs was higher in 0.5 and 1% chitosan treatments compared to J2 *in vitro*. This may be due to the fact that chitosan application; together with chitinase activity, cause nematode egg shells to deteriorate and then the spoilage inside the egg (Jatala, 1986; Moto and das Santos, 2016). The most effective concentration of liquid chitosan *in vitro* was found to be 2%, and the suppressive effect of J2 hatching from egg, egg mass and J2 mortality was determined as 73.9%, 78.6% and 74.0%, respectively. Khan et al. (2021) reported that chitosan caused 100% mortality of J2s within 36 h while Seo et al. (2014) also stated that chitosan caused J2 mortality approximately 95.8% after 48 h. The nematicidal effect of 2% liquid chitosan was similar to the nematicide Velum *in vitro*. This result shows that the liquid chitosan application is promising for controlling root knot nematodes. In previous studies, it was stated that chitin and chitosan applications could give successful results in controlling root knot nematodes (Ladner et al. 2008; Hussein et al. 2013; El Sayed and Mahdy 2015).

The suppressive effect of liquid chitosan was found to be lower than that of Velum application under controlled conditions. In addition, there was no significant difference between chitosan concentration applications in the number of gall and egg mass on tomato roots. However, when compared to negative control, 1 and 2% liquid chitosan applications were found to suppress *M. incognita* gall, egg mass and soil density by more than 55%. De-Jin et al. (2005) reported that galling caused by *M. incognita* decreased by 64% on tomato roots treated with chitin. Elkelany et al. (2020) showed that an 81.4% reduction in gall formation in eggplant roots in the experiment in which *M. incognita* eggs were inoculated 3 days after the application of chitosan to the soil. The results of the studies showed that the application of chitosan to the soil will be successful in the control against root knot nematodes. Organic materials, including chitin, can change the physicochemical properties of the soil, release nematicide compounds such as organic acids and nitrogen compounds (NH3), and can induce plant resistance by increasing antagonist microorganisms in the soil, resulting in the suppression of nematode populations (Oka, 2010; Castro et al., 2011). In addition, a hypersensitive reaction has been reported in plant tissues treated with chitosan (Hirano et al., 1999). This may prevent the feeding of nematode and cause its death (Kulikov et al., 2006). Researchers have shown that chitosan application in combination with different methods has higher success in the root knot nematode control. A significant reduction in nematode reproduction factor was found on tomatoes with chitin-enriched cattle manure vermicompost in soil infected with *M. incognita* compared to control (Castro et al., 2011). Chitosan combined with onion waste effectively controlled root-knot nematode disease and improved plant growth as well as the yield of eggplant (Ashif et al., 2017). In combination with botanicals (*Argemone mexicana* L., *Achyranthes aspera* L., and *Ricinus communis*), chitosan showed a synergistic effect against *M. incognita* on carrot as compared to chitosan

alone (Khan et al., 2021).

When the plant growth was evaluated, a significant difference was determined between 1 and 2% concentrations, and it was determined that 2% concentration adversely affected the tomato plant bıomass under controlled conditions. Mota and das Santos et al. (2016) reported that the application of chitosan alone to the soil adversely influences the shoot growth of tomatoes. A different study determined that the application of 1% chitosan to the soil in *Eustoma grandiflorum* Shinners plant gave rise to a positive effect on plant growth (Ohta et al., 2000). A positive effect of chitosan was observed on the growth of roots, shoots, and leaves of several crop plants (No et al., 2003; Nge et al., 2006; Khalil and Badawy, 2012). In this study, the concentration of 2% caused a very problematic growth in tomato plant and 1% concentration of chitosan application controlled *M. incognita* by 58%. This suggests that 1% concentration may be more effective in the control of *M. incognita*. The standard (1:1) concentrations of high- and low-molecular-weight chitosan polymers decreased over 70% gall, egg mass, soil J2 density and female individual number of *M. javanica* on tomato root (El Sayed and Mahdy, 2015).

Conclusion

The present study showed that efficient management of the nematode problem may be done by using chitosan. The increasing number of biotechnological studies on chitosan and its easy use in many areas due to its chemical and physical properties ensure a promising field of application for the future. However, more detailed studies need to be conducted under controlled and field conditions. The effect of chitosan application prior to planting should also be investigated. The chances of success can be increased by including chitosan polymers as an environmentally friendly component in an integrated nematode management system for sustainable agriculture. The benefit of the use of chitosan alone can be increased by combinations of different methods. Therefore, It is also necessary to look at the success of the use of chitosan together with fertilizers and nematophagous fungi in the control of root knot nematode.

Compliance with Ethical Standards

Conflict of interest

For this research article, the authors declared that they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. Authors have read and approved the final manuscript. The authors have verified that the text, figures and tables are original and that they have not been published before.

Consent for publication

The authors of this manuscript have agreed that the paper be published with your journal.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

References

Agrinos, (2014). HYT-C: Bioquitina. Retrieved from http://www.agrinos.com.mx/reporteco/upload/procesos/HYTc%20BioQuitina.pdf Accessed on October 29, 2014

Arslan, A. and Elekcioğlu, I.H. (2022). Biochemical and molecular identification of root-knot nematodes in greenhouse vegetable areas of Eastern Mediterranean Region (Turkey). Turkish Journal of Entomology, 46 (1): 115-127. Doi: http://dx.doi.org/10.16970/entoted.1055181

Asif, M., Ahmad, F., Tariq, M., Khan, A., Ansari, T., Khan, F., Siddiqui, A.M., (2017). Potential of chitosan alone and in combination with agricultural wastes against the root-knot nematode, *Meloidogyne incognita* infesting eggplant. Journal of Plant Protection Research, 57 (3): 288-295. Doi: https://doi.org/10.1515/jppr-2017-0041

Aydınlı, G. (2018). Detection of the root-knot nematode *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae) in vegetable fields of Samsun Province, Turkey. Turkish Journal of Entomology, 42 (3): 229-237. Doi: https://doi.org/10.16970/entoted.409941

Aydınlı, G., Mennan, S., Devran, Z., Širca, S., Urek, G. (2013). First report of the root-knot nematode *Meloidogyne ethiopic*a on tomato and cucumber in Turkey. Plant Disease, 97 (9): 1262-1262. Doi: https://doi.org/10.1094/PDIS-01-13-0019-PDN

Barker, K.R. (1985). Nematode extraction and bioassays. An advanced treatise on Meloidogyne, 2: 19-35. Retrieved from https://agris.fao.org/agris-search/search.do?recordID=US8743641

Berger, L.R.R., Stamford, T.C.H.M., Stamford, N.P. (2011). Perspectivas para ouso da quitosana na agricultura. Revista Iberoamericana de Polímeros, 12 (4): 195–215. Retrieved from https://reviberpol.files.wordpress.com/2019/07/2011-ramos.pdf

Castaneda-Alvarez, C. and Aballay, E. (2016). Rhizobacteria with nematicide aptitude: enzymes and compounds associated. World Journal of Microbiology and Biotechnology, 32 (12): 1-7. Doi: https://doi.org/10.1007/s11274-016-2165-6

Castro, L., Flores, L., Uribe, L. (2011). Efecto del vermicompost y quitina sobre el control de *Meloidogyne incognita* en tomate a nível de invernadero. Agronomía Costarricense, 35 (2): 21–32. ISSN:0377-9424 / 2011

Cetintas, R. and Cakmak, B. (2016). *Meloidogyne* species infesting tomatoes, cucumbers and eggplants grown in Kahramanmaraş Province, Turkey. Turkish Journal of Entomology, 40 (4): 355-364. Doi: http://dx.doi.org/10.16970/ted.40839

Collange, B., Navarrete, M., Peyre, G., Mateille, T., Tchamitchian, M. (2011). Root-knot nematode (Meloidogyne) management in vegetable crop production: The challenge of an agronomic system analysis. Crop protection, 30 (10): 1251-1262. Doi: https://doi.org/10.1016/j.cropro.2011.04.016

Coolen, W.A. and D'herde, C.J. (1972). A method for the quantitative extraction of nematodes from plant tissue. Publication of the State Nematology and Entomology Research Station, Merelbeke, Belgium, 77 p.

De-Jin, R., Suh, J., Park, R., Kim,Y. (2005). Effect of chitin compost and broth on biological control of *Meloidogyne incognita* on tomato (*Lycopersicon esculentum* Mill.). Nematology, 7 (1): 125–132. Retrieved from https://brill.com/view/journals/nemy/7/1/article-p125_13.xml

El Hadrami, A., Adam, L.R., El Hadrami, I., Daayf, F. (2010). Chitosan in plant protection. Marine Drugs, 8 (4): 968-987. Doi: https://doi.org/10.3390/md8040968

El-Ashry, R.M., El-Saadony, M.T., El-Sobki, A.E., El-Tahan, A.M., Al-Otaibi, S., El-Shehawi, A.M., Elshaer, N. (2022). Biological silicon nanoparticles maximize the efficiency of nematicides against biotic stress induced by *Meloidogyne incognit*a in eggplant. Saudi Journal of Biological Sciences, 29 (2): 920-932. Doi: https://doi.org/10.1016/j.sjbs.2021.10.013

Elkelany, U.S., El-Mougy, N.S., Abdel-Kader, M.M. (2020). Management of root-knot nematode *Meloidogyne incognita* of eggplant using some growth-promoting rhizobacteria and chitosan under greenhouse conditions. Egyptian Journal of Biological Pest Control, 30 (1): 1-7. Doi: https://doi.org/10.1186/s41938-020-00334-w

El-Sayed, S.M. and Mahdy, M.E. (2015). Effect of chitosan on root-knot nematode, *Meloidogyne javanica* on tomato plants. International Journal of ChemTech Research, 7 (4): 1985-1992. Retrieved from http://sphinxsai.com/2015/ch_vol7_no4/5/(1985-1992)%20V7N4.pdf

Fan, Z., Qin, Y., Liu, S., Xing, R., Yu, H., Li, K., Li, P. (2022). Fluoroalkenyl-Grafted Chitosan Oligosaccharide Derivative: An Exploration for Control Nematode *Meloidogyne incognita.* International Journal of Molecular Sciences, 23 (4): 2080. Doi: https://doi.org/10.3390/ijms23042080

Goverse, A. and Smant, G. (2014). The activation and suppression of plant innate immunity by parasitic nematodes. Annual review of phytopathology, 52: 243-265. Retrieved from https://www.annualreviews.org/doi/epdf/10.1146/annurev-phyto-102313-050118

Goy, R.C., Morais, S.T., Assis, O.B., et al. (2016). Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth. Revista Brasileira de Farmacognosia, 26 (1): 122-127. Doi: https://doi.org/10.1016/j.bjp.2015.09.010

Gürkan, B., Çetintaş, R., Gürkan, T. (2019). Determination of Root-Nematode species (Meloidogyne spp.) and some nematode population races in vegetable areas of Gaziantep and Osmaniye. KSU J. Agric Nat, 22 (Suppl 1): 113-124. (in Turkish with abstract in English). Doi: http://dx.doi.org/10.18016/ksutarimdoga.v22i49073.551240

Hashem, M., Moharam, A.M., Zaied, A.A., Saleh, F.E.M. (2010). Efficacy of essential oils in the control of cumin root rot disease caused by Fusarium spp. Crop Protection, 29 (10): 1111-1117. Doi: https://doi.org/10.1016/j.cropro.2010.04.020

Hirano, S., Nagamura, K., Zhang, M., Kim, S.K., Chung, B.G., Yoshikawa, M., Midorikawa, T. (1999). Chitosan staple fibers and their chemical modification with some aldehydes. Carbohydrate polymers, 38 (4): 293-298. Doi: https://doi.org/10.1016/S0144-8617(98)00126-X

Hussain, F., Shaukat, S.S., Abid, M., Usman, F., Akbar, M. (2013). Control of *Meloidogyne javanica* and *Fusarium solani* in chilli (*Capsicum annuum* L.) with the application of chitin. Pakistan Journal of Nematology, 31 (2): 165-170.

İmamoğlu, Ö. (2011). The use of chitosan as a biological control remedy. Turkish Bulletin of Hygiene & Experimental Biology, 68 (4): 215-222. Doi: http://dx.doi.org/10.5505/TurkHijyen.2011.55376

İmren, M., Özarslandan, A., Kasapoğlu, E.B., Toktay, H., Elekcioğlu, İ.H. (2014). Morphological and molecular identification of a new species *Meloidogyne artiellia* (Franklin) on wheat fauna in Turkey. Turkish Journal of Entomology, 38 (2): 189-196. Doi: https://doi.org/10.16970/ted.69266

Jaber, N., Al-Akayleh, F., Abdel-Rahem, R.A., Al-Remawi, M. (2021). Characterization ex vivo skin permeation and pharmacological studies of ibuprofen lysinate-chitosan-gold nanoparticles. Journal of Drug Delivery Science and Technology, 62: 102399. Doi: https://doi.org/10.1016/j.jddst.2021.102399

Jatala, P. (1986). Biological control of plant-parasitic nematodes. Annual Review Phytopathology, 24: 453–489. Retrieved from https://www.annualreviews.org/doi/pdf/10.1146/annurev.py.24.090186.002321

Karabörklü, S., Aydınlı, V., Dura, O. (2022). The potential of *Beauveria bassiana* and *Metarhizium an*isopliae in controlling the root-knot nematode *Meloidogyne incognita* in tomato and cucumber. Journal of Asia-Pacific Entomology, 25 (1): 101846. Doi: https://doi.org/10.1016/j.aspen.2021.101846

Khalil, M. S., and Badawy, M. E. (2012). Nematicidal activity of a biopolymer chitosan at different molecular weights against root-knot nematode, *Meloidogyne incognita*. Plant Protection Science, 48(4), 170-178. Doi: https://doi.org/10.17221/46/2011-PPS

Khan, A., Tariq, M., Ahmad, F., Mennan, S., Khan, F., Asif, M., Siddiqui, M. A. (2021). Assessment of nematicidal efficacy of chitosan in combination with botanicals against *Meloidogyne incognita* on carrot. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science, 71(4): 225-236. Doi: https://doi.org/10.1080/09064710.2021.1880620

Kulikov, S.N., Chirkov, S.N., Il'ina, A.V., Lopatin, S.A., Varlamov, V.P. (2006). Effect of the molecular weight of chitosan on its antiviral activity in plants. Applied Biochemistry and Microbiology, 42(2): 200-203. Doi: https://doi.org/10.1134/S0003683806020165

Kurtuluş, G. and Vardar, F. (2020). Properties of Chitosan, Application Areas, Effects on Plant Systems. International Journal of Advances in Engineering and Pure Sciences, 32 (3): 258-269. Doi: https://doi.org/10.7240/jeps.635430

Ladner, D.C., Tchounwou, P.B., Lawrence, G.W. (2008). Evaluation of the effect of Ecologic on root knot nematode, *Meloidogyne incognit*a, and tomato plant, *Lycopersicon esculentum*. International Journal of Environtment Research and Public Health, 5 (2): 104–110. Doi: https://doi.org/10.3390/ijerph5020104

Li, Y.C., Sun, X.J., Ge, Y.H., Wang, Y. (2009). Antifungal activity of chitosan on *Fusarium sulphureum* in relation to dry rot of potato tuber. Agriculture Science of China, 8 (5): 597-604. Doi: https://doi.org/10.1016/S1671-2927(08)60251-5

Liu, T., Wang, L., Duan, Y.X., Wang, X. (2008). Nematicidal activity of culture filtrate of *Beauveria bassiana* against *Meloidogyne hapla*. World Journal of Microbiology and Biotechnology, 24 (1): 113-118. Doi: https://doi.org/10.1007/s11274-007-9446-z

Malerba, M. and Cerana, R. (2015). Reactive oxygen and nitrogen species in defense/stress responses activated by chitosan in sycamore cultured cells. International Journal of Molecular Sciences, 16 (2): 3019-3034. Doi: https://doi.org/10.3390/ijms16023019

Misiha, P.K., Aly, A.Z., Mahrous, M.E., Tohamy, M.R.A. (2013). Effect of culture fılterates of three Trichoderma specıes, *Fusarium solani* and *Rhizoctonia solani* on egg hatchıng and juvenıle mortalıty of *Meloidogyne incognita* ın vıtro. Zagazig Journal of Agricultural Research, 40 (3): 1-9. Retrieved from https://www.academia.edu/41815854

Mota, L.C. and dos Santos, M.A. (2016). Chitin and chitosan on *Meloidogyne javanica* management and on chitinase activity in tomato plants. Tropical Plant Pathology, 41 (2): 84-90. Doi: https://doi.org/10.1007/s40858-016-0072-x

Mouniga, R., Anita, B., Shanthi, A., Lakshmanan, A., Karthikeyan, G. (2022). Phenol and antioxidant enzymatic activity in root knot nematode, *Meloidogyne incognita* infected tomato plants treated with chitosan nanoparticles. The Pharma Innovation Journal, 11 (4): 241-245. Doi: https://doi.org/10.22271/tpi.2022.v11.i4d.11754

Nico, A.I., Jimenez-Diaz, R.M., Castillo, P. (2004). Control of root-knot nematodes by composed agro-industrial wastes in potting mixtures. Crop Protection, 23 (7): 581–587. Doi: https://doi.org/10.1016/j.cropro.2003.11.005

Nige, K.L., New, N., Chandrkrachang, S., Stewens, W.F. (2006): Chitosan as a growth stimulator in orchid tissue culture. Plant Science, 170: 1185–1190. Retrieved from https://www.academia.edu/4173475/Chitosan_as_a_growth_stimulator_in_orchid_tissue_culture

No, H.K., Lee, K.S., Kim, I.D., Park, M.J., Kim, S.D., Meyers, S.P. (2003). Chitosan treatment affects yield, ascorbic acid content, and hardness of soybean sprouts. Journal of Food Science, 68: 680–685. Doi: https://doi.org/10.1111/j.1365-2621.2003.tb05731.x

Ntalli, N.G. and Caboni, P. (2012). Botanical nematicides in the mediterranean basin. Phytochemistry Reviews, 11 (4): 351-359. Doi: https://doi.org/10.1007/s11101-012-9254-4

Ohta, K., Atarashi, H., Shimatani, Y., Matsumoto, S., Asao, T., Hosoki, T. (2000). Effect of chitosan with or without nitrogen treatments on seedling growth in *Eustoma grandiflorum*. Journal of Japanese Society Horticultural Science, 69 (1): 63–65. Doi: https://doi.org/10.2503/jjshs.69.63

Oka, Y. (2010). Mechanisms of nematode suppression by organic soil amendments - a review. Applied Soil Ecology, 44 (2): 101–115. Doi: https://doi.org/10.1016/j.apsoil.2009.11.003

Rabea, E.I., El Badawy, M.T., Rogge, T.M., Stevens, C.V., Höfte, M., Steurbaut, W. (2005). Insecticidal and fungicidal activity of new synthesized chitosan derivatives. Pest Management Science, 61 (10): 951-960. Doi: https://doi.org/10.1002/ps.1085

Saad, A.M., Salem, H.M., El-Tahan, A.M., El-Saadony, M.T., Alotaibi, S.S., El-Shehawi, A.M., Swelum, A.A. (2022). Biological control: An effective approach against nematodes using black pepper plants (Piper nigrum L.). Saudi Journal of Biological Sciences, 29 (4): 2047-2055. Doi: https://doi.org/10.1016/j.sjbs.2022.01.004

Seid, A., Fininsa, C., Mekete, T., Decraemer, W., Wesemael, W.M. (2015). Tomato (*Solanum lycopersicum*) and root knot nematode (Meloidogyne spp.) a century old battle. Nematology, 17 (9): 195–1000. Retrieved from https://brill.com/view/journals/nemy/17/9/article-p995_1.xml

Seo, D.J., Nguyen, D.M.C., Park, R.D., Jung, W.J. (2014). Chitosan-cinnamon beads enhance suppressive activity against *Rhizoctonia solani* and *Meloidogyne incognita* in vitro. Microbial Pathogenesis, 66:44–47. Doi: https://doi.org/10.1016/j.micpath.2013.12.007

Shahidi, F. and Abuzaytoun, R. (2005). Chitin, chitosan, and coproducts: chemistry, production, applications, and health effects. Advances in Food Nutrition Research, 49 (4): 93-135.

Stevens, W.F. (2005). Chitin and Chitosan: Production and application research Asian Institute of Technology 1994-2004. Journal of Metals, Materials and Minerals, 15 (1): 73-81.

Struszczyk, H., Orlikowski, B.L., Skrzypczak, C. (2001). Chitosan in the control of soil-borne pathogens. Chitin Enzymology, 197-205.

Uysal, G., Söğüt, M.A., Elekçioğlu IH (2017). Identification and distribution of root-knot nematode species (Meloidogyne spp.) in vegetable growing areas of Lakes Region in Turkey. Turkish Journal of Entomology, 41 (1): 105-122. Doi: https://doi.org/10.16970/ted.91225