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Soil and Foliar Applications of Chitosan in the Control of Root-Knot Nematode in Tomato

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Abstract: In this research, the effect of soil, foliar and simultaneous soil+foliar applications of chitosan on gall and egg mass of root-knot nematode *Meloidogyne incognita* was investigated in tomato plants under controlled conditions (24±1°C, 60±5% RH). Fluopyram (0.16 ml/L) was used as positive control while only nematode-treated plants were considered as negative control. The study was set up in a randomized plot design with 5 replications for each application. The liquid suspension of chitosan diluted at 1% was used. In soil application, 5 ml was applied to each pot by using a graduated cylinder while in foliar application, the liquid suspension at 1% was applied using a portable hand sprayer until the solution was finished. The study was evaluated according to the 1-9 gall and egg mass index 60 days after inoculation of nematodes. The lowest gall (2.8) and egg mass (2.4) indexes were detected in the simultaneous soil+foliar application and took place in the same statistical group with the positive control nematicide. It was determined that the nematicidal effect of chitosan has increased in the case of combined soil and foliar applications. These results support the fact that chitosan applications are successful in the control of root-knot nematode.

Keywords: Chitosan, root-knot nematode, biological control, induced resistance

Domateste Kök Ur Nematodu Mücadelesinde Kitosanın Toprak ve Yaprak Uygulamaları

Öz: Bu çalışmada kontrollü koşullarda (24 ± 1°C, %60 ± 5 nem) domates bitkisinde kitosanın toprak, yaprak ve eş zamanlı toprak+yaprak uygulamalarının kök ur nematodu *Meloidogyne incognita*’nın gal ve yumurta paketi üzerindeki etkisi araştırılmıştır. Pozitif kontrol olarak Fluopyram (0.16 ml/L), negatif kontrol olarak sadece nematod uygulanan bitkiler kullanılmıştır. Çalışma her bir uygulama için tesadüf parselleri deneme deseninde 5 tekerrürlü olarak kurulmuştur. Çalışmada kitosanın %1 oranında seyreltilmiş sıvı süspansiyonu kullanılmıştır. Toprak uygulamasında her saksıya 5 ml mezür ile uygulanırken, yaprak uygulamasında ise hazırlanan %1’lik sıvı süspansiyonu portatif el püskürtücü kullanılarak çözelti bitene kadar uygulanmıştır. Çalışma nematod inokulasyonundan 60 gün sonra 1-9 gal ve yumurta paketi indeksine göre değerlendirilmiştir. En düşük gal (2.8) ve yumurta paketi (2.4) indeksi eş zamanlı toprak+yaprak uygulamasında tespit edilmiş ve pozitif kontrol nematisitle aynı istatistiki grupta yer almıştır. Kitosanın toprak ve yaprak uygulamasının beraber yapılması durumunda nematisidal etkinin arttığı belirlenmiştir. Bu sonuçlar kitosan uygulamalarının kök ur nematodu kontrolünde başarılı olduğunu desteklemektedir.

Anahtar Kelimeler: Kitosan, kök ur nematodu, biyolojik kontrol, uyarılmış dayanıklılık

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1. Introduction

There are more than 4100 plant parasitic nematode species identified so far (Zhu et al., 2022). Plant parasitic nematodes can cause an annual loss of 173 billion dollars (Zhu et al., 2022). Root-knot, cyst and root lesion nematodes constitute the most dangerous group. Root-knot nematodes (*Meloidogyne* spp.) have a wide host range and cause significant damage primarily to vegetable crops (Kiewnick and Sikora, 2006). The plants infected with root-knot nematodes also become more susceptible to bacterial and fungal diseases as well (Ashraf and Khan, 2010). *Meloidogyne incognita*, *M. arenaria*, *M. hapla* and *M. javanica* are the most important root-knot nematode species (Chen and Song, 2021).

Chemical nematicides and biopesticides are used for minimizing the losses arising from plant parasitic nematodes. However, the use of some nematicides is prohibited or restricted (Castaneda-Alvarez and Aballay, 2016). An additional disadvantage to the significant adverse effects of chemical nematicides on the ecosystem, environment, and human health has been the increase in resistance development due to intensive applications (Kiewnick and Sikora, 2006). There are a few biological agents used in the control of root-knot nematodes, the most common of which are *Paecilomyces lilacinum* and *Verticillium chlamydosporium* fungi. Nevertheless, their effectiveness is limited by their short-time persistence in the environment and instability under unfavorable conditions (Chandler et al., 2011). Therefore, environmentally friendly alternatives, biocontrol strains, sustainable dose formulations and new modes of action are needed (Forghani and Hajihassani 2020; Peiris, 2021).

Chitosan is a natural biopolymer produced by the deacetylation of chitin found in the outer shell and infungal cell walls of shrimps, shellfishe, lobsters or crabs. It was discovered by Henri Braconnot in 1811 (Chakraborty et al., 2020). Chitosan was reported to be environmentally friendly and biodegradable, inexpensive, easy to use, and able to control viruses, bacteria, fungi, insects, plant parasitic nematodes, and other pests locally and systemically (Anitha et al., 2014; Malerba and Cerana, 2016; Abd El-Aziz and Khalil, 2020; Alfy et al., 2020). Chitosan serves a variety of purposes in agriculture including fungicides, bio-stimulants, seed coating agents, soil conditioners, and even more. The soil remediation or its improvement using chitin and chitosan alleviates the symptoms of nematode damage (Mota and dos Santos, 2016; Fan et al., 2022). There are studies showing that the soil applications of chitin and chitosan reduce the density of root-knot nematodes gall, egg mass, and the 2nd instar larvae (L2) in the soil (Kalaiarasan et al., 2006; Ladner et al., 2008; El-Sayed and Mahdi, 2015). Moreover, foliar application has been widely used to control the development and spread of many diseases, including viral, bacterial, fungal diseases and pests (Rabea et al., 2003). It has been reported that the foliar applications of chitosan increase the production of some important metabolites (polyphenolics, flavonoids, lignin, and phytoalexins) in the plant and thus, induce the plant defense mechanism (Emami et al., 2017; Xoca-Orozco et al., 2017). In addition, chitosan-treated plants become less sensitive to the stresses caused by adverse conditions such as low or high temperature, salinity, and drought (Lizárraga-Paulín et al., 2011; Pongprayoon et al., 2013).

In the current study, it was intended to evaluate the effect of soil and foliar applications of chitosan on the gall and egg mass of the root-knot nematode *M. incognita* in tomato plants under controlled conditions.

2. Material and method

2.1. Materials

The *M. incognita* ISP isolate, which is defined morphologically and molecularly as root-knot nematode material and whose mass production continues under climate room conditions (24 ± 1°C and 60 ± 5% humidity), was used in the context of this study (Göze Özdemir et al., 2022a). The study was carried out on the Gulizar F1 tomato variety, which is known to be so susceptible to the root-knot nematode (Göze Özdemir et al., 2022b). The nematicide (Velum Prime®, Fluopyram) used as a positive control was purchased from Bayer Crop Science. The chitosan used in the study was obtained from the company, Kitinsan Agricultural Products and Industry Trade (Antalya, Türkiye).

2.2. Methods

Preparation of nematode inoculum: The nematode inoculum consisted of 1000 L2. After the roots of the mass-produced Tueza F1 tomato variety were washed in tap water, egg masses were removed from the roots under a stereo microscope and incubated in a Petri dish with water at 25 ± 2°C for three days. Three days later, L2s that had hatched from eggs were counted under the light microscope and adjusted to the number to use in experimentation and finally placed in 1 ml tubes (Göze Özdemir et al., 2022b).

The effects of soil and foliar applications of chitosan on the development of *M. incognita* in tomato roots: The study was set up in a climate room, the conditions of which were set at 24 ± 1°C and 60 ± 5 % RH, in plastic pots into the randomized plot design with 5 replications for each application. The study consisted of 5 applications; 1: Soil application, 2: Foliar application, 3: Simultaneous Soil + Foliar application, 4: Positive control and 5: Negative control.

Three-week-old tomato seedlings were transplanted into 14 cm diameter-plastic pots containing approximately 1500 g of sterile soil (68% sand, 21% silt and 11% clay). After one week, the inoculation of 1000 *M. incognita* L2/1ml per pot was conducted. Chitosan was applied one day after the nematode inoculation. In soil application, 5 ml of 1% liquid suspension was applied to each pot by using a graduated cylinder (Göze Özdemir et al., 2022c). In

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the foliar application, the previously prepared 1% liquid suspension was applied using a portable hand sprayer until the solution was completely finished (Sharathchandra et al., 2004). In the positive control nematicide (Velum Prime®) application, the field recommended dose was used, i.e., 0.16 ml/L per pot was applied. Only nematodes inoculated plants were used as negative control.

The experiment was terminated 60 days after nematode inoculation. Tomato plants from each application were carefully uprooted from the soil and washed with tap water. In evaluation, 1-9 gall (1: no gall, 2: 5 % root galling, 3: 6-10 %, 4: 11-18 %, 5: 19-25 %, 6: 26-50 %, 7: 51-65 %, 8: 66-75 %, 9: 76-100 %) and 1-9 egg mass index (1: no egg mass, 2: 1 or 2 egg masses, 3: 3-6 egg masses, 4: 7-10 egg masses, 5: 11-20 egg masses, 6: 21-30 egg masses, 7: 31-60 egg masses, 8: 61-100 egg masses, 9:100+ egg masses) were utilized (Göze Özdemir, 2022).

The data obtained as a result of the experiment were subjected to the SPSS (version 20.0) program statistical analysis and the analysis of variance (ANOVA) was performed to test the differences between the means. Means were compared with the Tukey HSD test ($P \leq 0.05$).

3. Results and Discussion

In the study, it was determined that soil, foliar and soil + foliar applications of the chitosan significantly reduced gall and egg mass formations by *M. incognita* in tomatoes as compared to the case of negative control. It was determined that the gall index found in the plants with chitosan soil application was lower than the one found in the plants with chitosan foliar application (Table 1). However, there was no statistical difference between soil and leaf applications in terms of egg mass index ($P \geq 0.05$). Among the chitosan applications, the highest suppressive effect on gall and egg mass was detected in the simultaneous soil + foliar application. In soil + foliar application, gall and egg mass index values were in the same statistical group as the positive control nematicide ($P \geq 0.05$).

This study revealed chitosan to possess great potential as a biocontrol of the root-knot nematode. Simultaneous soil and foliar application of chitosan were found to significantly suppress gall and egg mass in roots. In addition, the fact that this suppressive effect was found similar to the one that resulted from the nematicide is another important output of this research. The foliar application method of chitosan is the most used in plant disease control (Bittelli et al., 2001; Khan et al., 2002; Iriti et al., 2009). Chitosan was widely used through foliar application for the control of many diseases (viral, bacterial, fungal) and pests' growth, spreads, and development (Rabea et al., 2003; Faoro et al., 2008). This widespread use is thought to be due to the property of chitosan to trigger the plant's defense system locally and systemically (El Hadrami et al., 2010). Besides, previous studies have presented that chitosan is able to induce systemic resistance against nematodes and then can protect plants from root-knot nematode infection (Mouniga et al., 2022). Chitosan particles have been reported to activate multiple signaling pathways in the case when they are recognized by specific receptors on cell membranes or cells, enabling a range of physiological responses including the secretion of antinematode enzymes, production of antinematode compounds, cell wall reinforcement and hypersensitive response (HR)-mediated cell death (Hirano et al., 1999; Sato et al., 2019). This can prevent the nematodes from feeding leading to their deaths (Kulikov et al., 2006). Unlike leaves, chitosan has a different control effect on nematodes when applied to the soil. In addition to the resistance development, chitosan increases chitinolytic microorganisms' population growth in the soil (Mota and dos Santos, 2016). Therefore, it provides multiple defense mechanisms against plant parasitic nematodes. There are so many examples indicating that the soil application of chitosan is effective against root knot nematodes (de Jin et al., 2005; Kalaiarasan et al., 2006; Ladner et al., 2008; Korayem et al., 2008; Khalil and Badawy, 2012). El-Sayed and Mahdi (2015) found above 80% of a nematicidal effect of high molecular weighted chitosan's non-diluted standard concentration on *M. javanica*.

4. Conclusion

As a result, it has been determined that chitosan applications were successful in the control of root-knot nematode and the nematicidal effect increased in the case of combined soil and foliar application. However, there is an important necessity to prove the concept of results

Table 1. Effect of soil and foliar applications of chitosan on *Meloidogyne incognita* gal and egg mass in the tomato roots

Application	Gal Index Aver.+Standard Error	Egg Mass Index Aver.+Standard Error
Soil	4.2±0.3 b*	3.8±0.2 b
Foliar	5.8±0.4 c	5.0±0.3 b
Soil+Foliar	2.8±0.3 ab	2.4±0.4 a
Negative control (N+)	7.4±0.4 d	6.6±0.2 c
Positive control (Velum®)	2.0±0.3 a	1.6±0.2 a

*Lower case letters in the same column indicate statistical differences between treatments ($P \leq 0.05$).

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found within this study but in the field conditions. It is thought that the use of chitosan as a plant activator, which offers the opportunity for its use in IPM with environmental sustainability, will become disseminated. For that matter, it has been determined that the application of chitosan with environmentally friendly recycled wastes such as the soil regenerating materials (compost, biocontrol agents, mulching plants, shell residues) together can be important in controlling nematodes.

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Author Contributions

FGGO and IO designed the study, provided the materials and wrote the manuscript. TO, JCN and HC participated in the nematicidal effect bioassays and collected of data. All authors have read and approved the final manuscript. The authors have verified that the text, tables are original and that they have not been published before.

Conflict of Interest

As the authors of this study, we declare that we do not have any conflict of interest statement.

Ethics Committee Approval

As the authors of this study, we declare that we do not have any ethics committee approval.

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