Türk. entomol. derg., 2025, 49 (1): 19-26
DOI: http://dx.doi.org/10.16970/entoted.1621229

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

The effect of some biopesticides on the root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) damaging tomato plants[1]

Bazı biyopestisitlerin domateste zararlı kök-ur nematodu *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerine etkisi

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Abstract

In recent years, biopesticides have been widely investigated for the control of plant parasitic nematodes (PPNs). In this study, the effects of *Bacillus*-based biopesticides on the root-knot nematode (*Meloidogyne incognita*) (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) were investigated. The study was conducted at the Nematology Laboratory and Growth Room of the Department of Plant Protection, Faculty of Agriculture, Bolu Abant İzzet Baysal University in 2023. The efficacy of *Bacillus subtilis* (Biopesticide-I), *Bacillus licheniformis* strain RTI184 (Biopesticide-II) and *Paecilomyces lilacinus* strain 251 (Biopesticide-III) was compared with chemical nematicides and an untreated control. Treatments were applied at different times: (A) 5-7 days before transplanting, (B) at transplant by drench, (C) just after transplanting, and (D) 14 days after transplanting (DAT). The lowest gal formation was observed in Biopesticide-III + Nematicide-II (CD) treatment (0.40±0.24), followed by Nematicide-I (CD) (3.40±0.24) and Biopesticide-I (BD) (4.20±0.37), while the highest was observed in Biopesticide-I (CD) (5.00±0.31). The number of second-stage juveniles was significantly reduced by Biopesticide-III + Nematicide-II (CD) (99.76%), Nematicide-I (CD) (70.29%) and Biopesticide-I (BD) (57.36%). The results indicate that *Bacillus*-based biopesticides are effective in reducing root-knot nematode damage and can be used to control *M. incognita* in tomato plants.

Keywords: *Bacillus licheniformis* strain RTI184, *Bacillus subtilis*, biopesticide, nematicide, root-knot nematode

Öz

Son yıllarda, biyopestisitler bitki paraziti nematodların (BPN) kontrolü için yaygın olarak araştırılmaktadır. Bu çalışmada *Bacillus* bazlı biyopestisitlerin kök-ur nematodu *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerindeki etkileri incelenmiştir. Çalışma, 2023 yılında Bolu Abant İzzet Baysal Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nematoloji Laboratuvarı ve iklim odasında yürütülmüştür. Çalışmada *Bacillus subtilis* (Biyopestisit-I), *Bacillus licheniformis* suşu RTI184 (Biyopestisit-II) ve *Paecilomyces lilacinus* suşu 251'in (Biyopestisit-III) etkinliği, kimyasal nematisitler ve uygulama içermeyen bir kontrol ile karşılaştırılmıştır. Uygulamalar farklı zamanlarda gerçekleştirilmiştir: (A) dikimden 5-7 gün önce, (B) dikim sırasında daldırma yoluyla, (C) dikimden hemen sonra ve (D) dikimden 14 gün sonra (DAT). En düşük gal oluşumu Biyopestisit-III + Nematisit-II (CD) uygulamasında (0,40±0,24), ardından Nematisit-I (CD) (3,40±0,24) ve Biyopestisit-I (BD) (4,20±0,37) uygulamalarında gerçekleşmiş, en yüksek ise Biyopestisit-I (CD) (5,00±0,31) uygulamasında gözlenmiştir. İkinci dönem larva sayıları Biyopestisit-III + Nematisit-II (CD) (%99,76), Nematisit-I (CD) (%70,29) ve Biyopestisit-I (BD) (%57,36) ile önemli ölçüde azalmıştır. Sonuçlar, *Bacillus* bazlı biyopestisitlerin kök-ur nematodu zararını azaltmada etkili olduğunu ve domates bitkilerinde *M. incognita*'nın mücadelesinde kullanılabileceğini göstermektedir.

Anahtar sözcükler: *Bacillus licheniformis* RTI184 suşu, *Bacillus subtilis*, biyopestisit, nematisit, kök-ur nematodu

[1] This study is derived from the MSc thesis of the first author.

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Received (Alınış): 16.01.2025 Accepted (Kabul ediliş): 04.04.2025 Published Online (Çevrimiçi Yayın Tarihi): 05.04.2025

Introduction

The tomato is among the most lucrative vegetable crops, widely cultivated in tropical, subtropical, and temperate climates globally (Naika et al., 2005). This vital crop experiences substantial productivity losses attributable to a number of diseases, including bacterial, fungal, viral, and nematode infections (Netscher & Sikora, 1990). Plant-parasitic nematodes (PPNs) represent a significant threat to global agricultural output. The damage inflicted by PPNs is projected to lead to a significant 12.3% reduction in global yield, amounting to approximately $157 billion each year. The current taxonomy recognizes approximately 4,100 species of PPNs, with root-knot nematodes (RKNs) and cereal cyst nematodes (CCNs) being acknowledged as significant plant diseases, whereas other species exhibit a more restricted host range (Singh et al., 2015).

Among the PPNs, root-knot nematodes (*Meloidogyne* spp.) are especially infamous, causing enormous agricultural losses estimated at $100 billion annually (Elling, 2013). More than 100 species of RKNs have been found, with four species *Meloidogyne incognita* (Kofoid & White,1919) Chitwood,1949, *Meloidogyne javanica* (Treub,1885) Chitwood,1949, *Meloidogyne hapla* Chitwood, 1949 and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) predominating and causing up to 90% damage to infected plants (Hunt & Handoo, 2009; Lunt et al., 2014; Khan et al., 2023). These nematodes are obligate, sedentary parasites with a wide host range, able to infect more than 5,000 plant species, including vegetables, fruits, field crops, and ornamental plants (Blok et al., 2008). These organisms are very hard to manage as they have a short life cycle, high reproductive potential and they attack the roots of the plants continuously (Sikora et al., 1992).

Conventional control methods for RKNs include soil solarization, non-fumigant and fumigant nematicides, and planting RKN-resistant varieties (Giannakou & Anastasiadis, 2005; Jordan, 2018). The prevalence and regularity of nematicide use over the past decades have created substantial disadvantages, as these substances are highly harmful both to human health and the soil ecosystem (Rajasekharan et al., 2020). Consequently, there is an imperative for the formulation of effective, ecologically sustainable alternative strategies for the control of RKNs. Biological control of pests is one of the most promising ways to control PPNs (Hallmann et al., 2009; Collange et al., 2011).

Products containing antagonistic microorganisms, usually bacteria or fungi, are generally referred to as bioproducts or biopesticides and are commonly known as microbial pesticides. Biopesticides are considered an integral part of integrated pest management (IPM) strategies (Arora et al., 2000). Recent international research investigated the potential of antagonistic microorganisms to alleviate the adverse effects of PPNs (Zheng et al., 2016; Abd- Elgawad et al., 2021). Many microorganisms proved to be highly effective as biological control agents against RKNs (Kerry, 2000). Investigations have focused on bacterial strains of the genera *Bacillus*, *Pseudomonas*, and *Pasteuria* for the control of RKNs (Aballay et al., 2012; Mahesha et al., 2017).

This study aims to evaluate the nematicidal efficacy of locally derived commercial biopesticides against *M. incognita*, the predominant species of RKN affecting tomato plants in controlled environments.

Materials and Methods

Nematode population

The RKN species, *M. incognita*, was used in the experiment. The cultures of the nematodes were established from different egg masses and were then maintained on a tomato cultivar in a controlled growth room at the Faculty of Agriculture, Abant İzzet Baysal University. During the study, the environmental temperature was controlled at 25 ± 2°C, with relative humidity kept at %60±10. Infected roots were carefully cleaned to remove attached dirt. Egg masses from the infected plants were collected with care and immersed in distilled water. These were then placed in a BOD incubator at 28 ± 2°C to obtain the second-stage juveniles (J2). The juvenile suspension was calibrated to a final concentration of 100 juveniles per milliliter of distilled water.

Experimental design and set up

The experimental setup followed a completely randomized design, featuring five treatments with five replications each. The experiments were conducted in 500 cc plastic pots. The soil mixture, composed of 75% sand and 25% peat, was sterilized in an autoclave at 121°C. Three-week-old sensitive tomato seedlings (variety Falcon) were planted in each pot for each treatment. The J2 inoculum, standardized at a rate of 500 J2 per pot, was carefully added into two wells near the plant roots using a 5 ml micropipette, immediately before the biopesticide and nematicide treatments, according to the experimental design. The biopesticides and nematicides listed in the table below have been used in the experiment (Table 1).

Table 1. Biopesticides and nematicides used in the experiment

Code	Active substance	Trade name of product	Company (Türkiye)
Biopesticides-I	*Bacillus subtilis*	Basuka	Ecobio Agriculture Products Ind. Ltd. Co.
Biopesticides-II	*Bacillus licheniformis* (RTI184)	Accudo	FMC Türkiye Industrial Products Ind. Ltd. Co.
Biopesticides-III	*Paecilomyces lilacinus* strain 251	Bioact DC 216	Bayer Türk Chemistry Ind. Ltd. Co.
Nematicide- I	Abamectin 20 g/L	Tervigo 20 SC	Syngenta Agriculture Ind. Anon. Co.
Nematicide- II	Fluopyram 400 g/L	Velum Prime SC 400	Bayer Türk Chemistry Ind. Ltd. Co.

In the study investigating the effect of some biopesticides on the reproduction of the RKN species *M. incognita*, the treatments were applied at different times to assess their impact on various stages of the nematode's life cycle (Table 2).

Table 2. Application times of biopesticides and nematicides treatments

Timing/Application code	A	B	C	D
	5-7 days before transplantation to soil without plant	At transplantation by dipping	Just after transplantation	14 days after transplantation (14DAT)

Immediately after inoculation, the biopesticides and nematicides used in the trial were applied at recommended doses, with consideration that tomato seedlings must be planted at 1500 plants/da according to the (Table 3). After application, the pots were irrigated to enhance the effect.

Table 3. Used treatments in the experiment with used product formulation, application rates and and times

No	Treatments	Form.	Rate (ml/hL or g/hL)	Time
1	Biopesticides-I	SG	1 L/ha (0.05 L/1000 plant)	ABD
2	Biopesticides-I	SG	1 L/ha	BD
3	Biopesticides-I	SG	1 L/ ha	CD
4	Biopesticides-II	SC	1 L/ha + 0.5L/ha	CD
5	Biopesticides-III + Nematicide-II	DC-SC	0.75 L/ha+0.6L/ha	CD
6	Nematicide-I	SC	4 L /ha	CD
7	Control (+) (nematode Applied)	NA	NA	NA
8	Control (-)	NA	NA	NA

* SG: water soluble granules, DC: dispersible concentrates: SC: suspension concentrate, NA: Non-application.

Evaluation of the trial

Following a period of eight weeks from the commencement of the experiment, the tomato plants were ready for harvesting. The plants were harvested by cutting them at ground level, and their roots were extracted from the soil with great care. The roots were then washed gently under a stream of running water to remove any adhering soil particles. Subsequent to this process, the fresh and dry weights of the roots were meticulously measured and recorded. The severity of root galling, evaluated using a scale ranging from 0 to 10 as per Zeck (1971), provided insights into the extent of damage caused by nematode infestation. The improved Baermann funnel method (Hooper, 1986) was used to determine the population density of *M. incognita*. Finally, the collected second-stage juveniles (J2) were counted under an inverted microscope.

Statistical analysis

The SPSS software (version 15.00; SPSS, Chicago, IL, USA) was used to statistically evaluate the experimental data. Analysis of variance (ANOVA) was employed to assess the significance of differences among the various parameters measured in the experiment. ANOVA enabled the comparison of means across many treatment and control groups. The Duncan test was used for post-hoc mean comparisons at a significance threshold of p<0.05 to identify homogeneous groupings.

Results

The impact of the treatments on the reproduction of the nematode

The results demonstrated that all treatments considerably diminished tomato root galling in comparison to the untreated control. The combination of Biopesticides-III with Nematicide-II exhibited the greatest efficacy, diminishing root galling by 93.81%, closely succeeded by Nematicide-I (CD), which achieved a reduction of 51.43%. Biopesticide-I (BD), Biopesticide-I (ABD), and Biopesticide-II (CD) shown significant decreases in root galling of 40.00%, 33.69%, and 33.33%, respectively (Table 4).

Moreover, all tested treatments significantly decreased the J2 population in the soil at recommended dosage rates compared to the untreated control. Biopesticides-III + Nematicide-II and Nematicide-I were particularly effective, reducing J2 in the soil by 99.76% and 70.29%, respectively. Biopesticides-I (BD), Biopesticides-I (CD), and Biopesticides-I (ABD) followed with reductions of 57.36%, 49.66%, and 42.31%, respectively (Table 4).

Table 4. The impact of biopesticides and nematicides on the root-gall index and second-stage juveniles (J2)

Treatments	Time	Root-gall index (Mean ± SE)*	Mean J2 /250 g soil (Mean ± SE)	Decrease in galls over control (%)	Decrease in J2 over control (%)
Biopesticides-I	ABD	4.60 ± 0.24^{c}	954.00 ± 117.32^{cd}	33.69	42.31
Biopesticides-I	BD	4.20 ± 0.37^{bc}	712.00 ± 133.01^{bc}	40.00	57.36
Biopesticides-I	CD	5.00 ± 0.31^{c}	832.00 ± 54.99^{cd}	27.14	49.66
Biopesticides-II	CD	4.60 ± 0.24^{c}	1250.00 ± 96.85^{e}	33.33	24.18
Biopesticides-III + Nematicide-II	CD	0.40 ± 0.24^{a}	4.00 ± 2.44^{a}	93.81	99.76
Nematicide-I	CD	3.40 ± 0.24^{b}	490.00 ± 53.85^{b}	51.43	70.29
Control (+) applied nematod	NA	7.00 ± 0.31^{d}	1656.00 ± 62.81^{f}	0.00	0.00
Control (-)	NA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00	0.00

* Each value is the mean of five replicates. Values in each column labeled with the same letter(s) indicate no statistically significant differences within the acceptable significance range (p<0.05), according to Duncan's multiple-range analysis.

Furthermore, the combined treatments of Biopesticides-III + Nematicide-II demonstrated more significant efficacy compared to single treatments with Biopesticides-I and Biopesticides-II. Notably, transplant drench applications of *Bacillus*-based biopesticides proved more effective than other biopesticide treatments. These findings underscore the potential of integrated pest management strategies involving both biological products and nematicides for effective control of RKNs in tomato cultivation. Such integrated approaches hold promises for sustainable nematode management practices in agriculture.

The impact of treatments on plant growth parameters

According to the findings, the presence of nematode populations had a notable negative impact on plant growth indices compared to the nematode-free control. Significant increases in plant height were observed in treatments involving Biopesticides-III + Nematicide-II and Biopesticides-II. Conversely, plant height was significantly reduced at Biopesticides-I (ABD) and Biopesticides-I (BD) treatments compared to the nematode-free control. Other treatments did not differ significantly from the untreated nematode inoculated plants in terms of their effect on plant height (Table 5).

Regarding root weight, all treatments showed no significant difference from the control, except for Nematicide-I and Biopesticides-III + Nematicide-II, which significantly increased root weight compared to the control (+). The maximum biometric parameters were recorded in plants treated with Biopesticides-III + Nematicide-II (Table 5).

When compared to untreated inoculated plants, neither Biopesticides-I nor Biopesticides-II significantly impacted the growth indices of tomato plants. These results underscore the differential effects of various treatments on tomato plant growth under nematode-infested conditions, highlighting the potential benefits of integrated approaches such as Biopesticides-III + Nematicide-II in mitigating the negative impact of nematode infestations on plant development and productivity (Table 5).

Table 5. The impact biopesticides and nematicides on the growth parameters of tomato plants

Treatments	Time	Plant height (Mean ± SE)*	Root fresh weight (Mean ± SE)	Root dry weight (Mean ± SE)
Biopesticides-I	ABD	30.20±0.58[a]	24.05±1.98[ab]	2.34±0.16[ab]
Biopesticides-I	BD	29.40±2.06[a]	21.55±1.29[ab]	2.09±0.20[a]
Biopesticides-I	CD	39.80±2.05[bc]	20.39±0.99[a]	1.90±0.23[a]
Biopesticides-II	CD	42.40±1.43[cd]	22.45±2.07[ab]	2.15±0.35[a]
Biopesticides-III + Nematicide-II	CD	45.40±0.92[d]	43.99±2.80[c]	4.69±0.29[c]
Nematicide-I	CD	37.80±1.62[b]	44.24±4.56[c]	4.95±0.48[c]
Control (+) applied nematod	NA	26.60±0.50[a]	18.58±1.94[a]	1.89±0.23[a]
Control (-)	NA	50.20±0.86[e]	49.06±1.10[c]	5.24±0.13[c]

* Each value is the mean of five replicates. Values in each column labeled with the same letter(s) indicate no statistically significant differences within the acceptable significance range ($p<0.05$), according to Duncan's multiple-range analysis.

Discussion

Globally, agricultural practices have traditionally relied on chemical nematicides to control RKNs because of their rapid and often effective control (Burkett-Cadena et al., 2008). However, concerns over their environmental impacts, including soil toxicity and effects on non-target organisms, have caused the development of stringent regulatory controls and total bans on several compounds over the past two decades (Mukhtar et al., 2013). The situation has been marked by a dire need for sustainable options that enhance the current Integrated Pest Management (IPM) systems.

Research has demonstrated that rhizobacteria belonging to the *Bacillus* genus function efficiently as biological control agents for RKNs. These bacteria address PPNs through several mechanisms, such as the synthesis of volatile organic compounds (VOCs), which impede nematode growth and attract beneficial microorganisms. The fact that these organisms can induce systemic resistance in plants makes them a very interesting environmentally friendly substitute for chemical treatments (Siddiqui & Mahmood, 1999).

The current study was conducted to assess the efficiency of various *Bacillus*-based bioproducts in managing *Meloidogyne incognita* (Kofoid and White) Chitwood, 1949 (Tylenchida: Meloidogynidae) under controlled growth room conditions. The utilization of biopesticides, specifically Biopesticides-I (BD), Biopesticides-I (ABD), and Biopesticides-II (CD), has demonstrated a significant decrease in root galling, with observed reductions between 33.33% and 40.00%. Our findings are consistent with prior research demonstrating various RKN control experiments conducted by rhizobacteria related to the *Bacillus* genus. For example, Freitas et al. (2005) reported that *B. cereus* isolates to control *M. javanica* observed reduce the intensity of galls approximately 55% when applied via tomato seeds. Colagiero et al. (2018) reported that *in vitro* tests using *B. cereus* and *B. licheniformis* against *M. incognita* showed that *B. licheniformis* significantly reduced the ability of *M. incognita* second-stage juveniles to infect tomato roots. Ramalakshmi et al. (2020) reported that the biocontrol potential of 2 native *B. thurungiensis* was evaluated for their effective control of *M. incognita* under greenhouse conditions. The final nematode population in the soil was 52.4% and 59.5%, and gall index values were reduced by 46.7% and 66.7%. Xiao et al. (2018) reported that *Bacillus cereus* strain Jdm1 was tested for activity as a biocontrol agent against *M. incognita*. The root galling severity decreased 43%, with improved growth compared to control plants. Hussain et al. (2020) reported that tomato roots in combined application of culture filtrate of *B. subtilis* was effective in reducing the root galling by 58.79%. Huang et al. (2020) reported that *Bacillus* genus bacteria reduced the second-stage juveniles of RKN rates to 64.58%. Yanyan et al. (2023) reported that treatments with cultures of *Bacillus firmus* strain YB-1503 reduced root gall index by 65.8% and increased plant growth, a significant improvement over the control. Niazi (2024) reported that the combination of *B. subtilis* and *Pseudomonas putida* cultures reduced the effects of *M. incognita* on tomato plants, reducing the nematode population by 50% and gall formation by 60%.

Although the results under controlled conditions are promising, the extrapolation of biocontrol efficacy from the laboratory to the field is problematic (Meyer, 2003; Tian et al., 2007). The differences between pot trials and field results can be explained by the soil texture, climatic conditions, and microbial competition. Meyer (2003) and Tian et al. (2007) highlighted the importance of understanding these complex interactions in order to improve the applicability and efficacy of *Bacillus*-based biopesticides in real agricultural scenarios.

Furthermore, comparisons with chemical nematicides show that chemical treatments gave higher levels of suppression than biopesticides under the same conditions. In addition, the combination of the fungal biopesticide *P. lilacinus* strain 251 and the nematicide fluopyram also achieved very high levels of control. This suggests that biologicals alone may not provide sufficient efficacy and that their use in combination with chemical nematicides is important to ensure higher yields and effective pest management. Therefore, the use of biopesticides and chemical nematicides in an integrated sustainable pest management system should be considered.

In conclusion, the study showed that *Bacillus*-based biopesticides, including *B. subtilis* and *B. licheniformis*, can effectively reduce *M. incognita* population densities and can be used in insecticide-based management strategies. Although chemical nematicides provide better control, these biopesticides are safe for plants and the environment. Further research is needed to improve their efficacy under field conditions, considering factors such as soil composition, climate and microbial interactions.

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