Türk. entomol. derg., 2024, 48 (2): 195-204
DOI: http://dx.doi.org/10.16970/entoted.1452672

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

The effectiveness of some rhizobacteria on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) in cucumber plants[1]

Bazı rizobakterilerin hıyar bitkisinde *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae)'ya karşı etkinliği

Deniz YAŞAR[2] **Galip KAŞKAVALCI[2*]**

Abstract

In this study, the possibilities of using 3 specific rhizobacteria isolates for the control of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) in cucumber plants of Beith alpha cultivar were investigated in 2023. The variables of the climate chamber experiment were seedling and seed treatments of the specific bacterial isolates and the QST713 (Serenade®) commercial isolate, nematode (1500 J2/pot) and non-nematode treatments, as well as positive and negative control treatments. As a result of the study, when the rate of root galling on cucumber roots was determined according to the Zeck scale, the most successful treatment in decreasing root galling was the seedling treatment of KD29 isolate (2.64), while the highest rate of root galling was observed in the positive control (6.27). When the bacterial treatments were compared with the positive control, it was observed that all bacterial treatments had a decreasing effect on the number of egg mass. When the effects of the treatments on the reproduction rate of the J2 population were analyzed, it was found that seedling treatments of isolate KD238 (0.69) and commercial isolate QST713 (0.86) had a decreasing effect on the J2 reproduction rate in the soil. As a result of the laboratory experiment, it was determined that KD157, KD238 and KD29 isolates had 42.25, 33.98 and 27.77% mortality effect on J2s after 96 hours, respectively. However, especially considering the decrease in the J2 population in the soil, the amount of root growth and the decrease in the number of egg mass, these bacteria stimulate the induced systemic resistance (ISR).

Keywords: *Bacillus thuringiensis*, *Pantoea* spp., PGPR, *Pseudomonas* spp., root-knot nematodes

Öz

Bu çalışmada, 3 adet özgün rizobakteri izolatının, Beith alpha çeşidi hıyar bitkisinde *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) ile mücadelede kullanım olanakları 2023 yılında araştırılmıştır. Yapılan çalışmalarda bakteri uygulamalarının *M. incognita*'ya karşı etkinliği iklim odası ve laboratuvar denemesi yapılarak değerlendirilmiştir. İklim odası denemesinin karakterlerini özgün bakteri izolatlarının ve QST713 (Serenade®) ticari izolatının fide ve tohum kaplama uygulamaları, bu uygulamaların nematodlu (1500 J2/saksı) ve nematodsuz uygulamaları, pozitif ve negatif kontrol uygulamaları oluşturmuştur. Deneme sonunda, hıyar köklerindeki urlanma oranı Zeck skalasına göre değerlendirildiğinde, köklerdeki urlanma miktarını azaltma konusunda en başarılı uygulama KD29 izolatının (2.64) fide uygulaması olurken, en yüksek urlanma miktarı pozitif kontrolde (6.27) görülmüştür. Bakteri uygulamaları pozitif kontrol ile kıyaslandığında, yumurta kümesi oluşumu üzerinde tüm bakteri uygulamalarının azaltıcı etkiye sahip olduğu görülmüştür. Yapılan uygulamaların, J2 popülasyonunun üreme oranı üzerindeki etkileri araştırıldığında, KD238 (0.69) izolatı ve QST713 (0.86) ticari izolatının fide uygulamalarının, topraktaki J2 üreme oranı üzerinde azaltıcı etkiye sahip olduğu saptanmıştır. Yapılan laboratuvar denemesi sonucunda KD157, KD238 ve KD29 izolatlarının 96 saat sonunda J2'ler üzerinde sırasıyla %42.25, 33.98 ve 27.77 oranında öldürücü etkiye sahip olduğu saptanmıştır. Ancak özellikle topraktaki J2 popülasyonunun azalması, kökte oluşan ur miktarı ve yumurta kümesi sayılarındaki azalma göz önüne alındığında, bu bakterilerin uyarılmış sistemik dayanıklılığı (ISR) teşvik ettiği düşünülmektedir.

Anahtar sözcükler: *Bacillus thuringiensis*, *Pantoea* spp., PGPR, *Pseudomonas* spp., kök-ur nematodları

[1] This article has been drawn up from the first author's Master Science thesis.

[2] Ege University, Faculty of Agriculture, Department of Plant Protection, 35100, Bornova, İzmir, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: galip.kaskavalci@ege.edu.tr
Received (Alınış): 15.03.2024 Accepted (Kabul ediliş): 01.05.2024 Published Online (Çevrimiçi Yayın Tarihi): 16.05.2024

Introduction

Cucumber is an annual plant species that grows in warm to subtropical climates. Cucumber, *Cucumis sativus* L. is a member of the Cucurbitaceae (Cucurbitales) family and is cultivated in most parts of the world as a rich source of vitamins and minerals. Cucumber is the most cultivated vegetable after tomato, watermelon, and onion, with a production of around 1.9 million tons in Türkiye (TÜİK, 2022). According to FAO statistics, cucumber production was 1.890.160 tons worldwide in 2021. China has the highest cucumber production in the world with 75.547.733 tons. Türkiye is the second cucumber growing country, followed by China (FAO, 2021). However, farmers need to manage important pests and diseases that prevent them from achieving the expected yields in cucumber production. Root-root nematodes, *Meloidogyne* spp. Göldi, 1892 (Nematoda: Meloidogynidae) have an important place among these pests. Root-knot nematodes spend part of their life in the soil as egg or J2 form. As a result of the feeding of J2s on the roots, root-knot formation is observed. The formed knots block the plant's absorbance of water and nutrients from the soil. Consequently, the plant becomes stunted, growth and development are impaired and fruit quality decreases (Echeverrigaray et al., 2010).

When necessary, precautions are not taken in agricultural areas where root-knot nematodes are contaminated with vegetables, crop losses depend on the intensity of the pest and the type and sensitivity of the plant cultivated. The crop losses can generally reach up to 15-85% in vegetables (Anonymous, 2008), and 16-47% in cucumber plants grown under greenhouse conditions (Netscher & Sikora, 1990). Different management methods are used to minimize the damage of plant parasitic nematodes that cause such crop losses in agricultural areas. Among these methods, nematicides has an important place in chemical control, which has a critical role in the global market with an annual share of 1.3 billion dollars (Oka, 2020). However, although chemical control is the first choice of farmers due to its ease of application and cheapness, it does not produce long-term and long-lasting results on plant parasitic nematodes.

On the other hand, biological management is one of the alternative control methods that have been intensively studied in recent years. In biological control against root-knot nematodes, bacteria living in the rhizosphere, called plant growth-promoting rhizobacteria (PGPR), have an important potential as biological control agent (Paul & Lade, 2014). The mechanism of action of this group, called plant growth-promoting rhizobacteria (PGPR), is quite broad. These mechanisms can be classified as direct antagonistic effect and indirect effect. Direct antagonistic effects include inhibition of nematode populations by producing toxins, enzymes, and other metabolic components, while indirect effects include activating mechanisms between the plant and nematode (promotion of systemic resistance), competition for nutrients, and reducing populations by regulating nematode behavior. The toxins produced by rhizobacteria inhibit nematode hatching, suppress their reproduction, or directly cause their death (Tian et al., 2007).

PGPR bacteria, which have an important place among biological control agents and are also used as biopreparations, are an alternative that does not cause residue problems compared to chemical control and supports plant growth. In this study, it was aimed to analyze the possibilities of using specific rhizobacteria preparations in the control of root-knot nematodes *in vivo* and *in vitro* trials.

Materials and Methods

Nematode culture

The root-knot nematode population used in the experiment was obtained by reproduction of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Nematoda: Meloidogynidae) pure culture in Nematology climate chamber. Beith alpha cucumber variety, which is sensitive to root-knot nematode, was used for population multiplication. Seedlings were transplanted into 1:1 ratio of sand and clay sand and soil mixture in half-liter plastic pots at the 3-leaf stage. 1500 *M. incognita* J2 were introduced through holes drilled near the root collar of the plants. The pure culture was grown in a climate chamber at 16:8

photoperiod and 27±3ºC. Plants were harvested 8 weeks after nematode inoculation. Plant roots were gently cleaned from the soil and rinsed. Egg masses on the roots were collected and J2 were obtained by using the improved Baermann funnel technique. The J2 were kept at +4ºC to be used in the experiments.

Preparation of bacterial suspensions

Three specific bacterial isolates (KD29, KD157 and KD238), which were found to be the most successful as a result of *in vitro* PGPR tests, were used in the experiment and these isolates were obtained from the collection of Prof. Dr. Hatice Özaktan from Ege University Faculty of Agriculture, Department of Plant Protection, Bacteriology Laboratory. Serenade® (*Bacillus subtilis* QST713) was used as a control to compare the effectiveness of the bacteria. Bacteria grown on King B medium for 24-48 hours at 24ºC were suspended by adding sterile distilled water. The suspensions obtained for each specific bacterial isolate were adjusted to OD_{600}=0.1 (1x 10^8 cfu/ml) using a spectrophotometer (Akbaba & Özaktan, 2018). Serenade® (*Bacillus subtilis* QST713) commercial isolate was used at a recommended dose of 1000 ml/100 l.

Identification of bacteria

Bacteria were cultured on King B medium, and pure colonies grown at 25ºC for 24 hours were suspended in sterile water in eppendorf tubes. Genomic DNA was then isolated by boiling the prepared suspensions at 95°C for 15 minutes. The DNA obtained was PCRed with 27F/1492F primers amplifying the 16S rRNA region. The PCR products obtained were sent for two-way sequence analysis through service procurement. The sequences of the Reverse primer of the incoming sequence files were translated into Forward primer by reverse translation. Both sequence files were aligned according to the ClusterW method with the help of the MEGAX program. Then, the alignment result was compared with the help of BioEdit program, and the Contig Sequence was created by removing unnecessary SNPs and completing the missing SNPs. The obtained Contig sequence was definitively diagnosed with the help of the BLAST program on the NCBI website (Akbaba & Özaktan, 2018).

Effect of bacterial extracts on J2 immobility and mortality *in vitro* trials

The experiment was achieved in the Nematology Laboratory of Ege University, Faculty of Agriculture, Department of Plant Protection between February 23 and May 8, 2023. This experiment was carried out to observe whether suspensions obtained from specific bacterial isolates have mortality effects on J2s.

The experiment was established with 6 variables, 4 replicates and repeated twice. In the experiment, 4 well plates with 12 wells each were used. In each well, 100 newly hatched J2 and 1 ml of bacterial suspension were added by micropipette. Since the suspensions contained sterile distilled water, in the experimental conditions sterile distilled water was added to see if this treatment influenced the J2 and 1 ml when applied to the wells. As a negative control, 1 ml of tap water was used. All plates were kept in a shaker (Biosan PSU-20i) at 105 rpm for 96 hours at room temperature of 25±3ºC to avoid bacteria settling to the bottom. Throughout the study, nematode mortality rates were recorded regularly at 24, 48, 72 and 96 hours. The mortality of nematodes was checked by touching the nematode needle and nematodes that did not move were recorded as dead.

Effect of rhizobacteria on *Meloidogyne incognita* J2 *in vivo* trials

This study was conducted between 2022 and 2023 in the Nematology Climate Room of Ege University, Faculty of Agriculture, Department of Plant Protection. Cucumber (*Cucumis sativus* L.) seeds of Beith Alpha cultivar and seedlings at 3-leaf stage were used in the experiment. Half-liter pots used in the experiment were filled with a 1:1 mixture of sand and clay soil. The plants used in the study were grown in controlled conditions at 16:8 photoperiod and 27±3ºC in the climate room. The pot experiment was setup with 2 replications with 6 replicates with 18 variables. The experimental variables were seed treatment and soil drenching application of each bacterial isolate (KD29, KD157 and KD238) and Serenade® (*Bacillus*

subtilis QST713) commercial isolate, nematode and non-nematode treatments of these treatments, as well as positive control (N+) and negative control (N-) treatments. In half of the variables treated with positive control and bacteria, J2 of *M. incognita* were given to each plant in the amount of 1500 J2 through 5 cm deep holes drilled around the root collar from two different directions. The other half were not given nematode treatment to compare the effectiveness of bacteria and plant growth with the negative control.

Bacterial treatments were applied as follows:

1-Seed treatment

Bacteria grown on King B medium for 24-48 hours at 24ºC were suspended by adding carboxymethyl cellulose (CMC, 1% v/v). The suspensions obtained for each specific bacterial isolate were adjusted to OD_{600}=0.1 (1x 10^8 cfu/ml) using a spectrophotometer. Serenade® (*Bacillus subtilis* QST713) was applied at a recommended dose of 1000 ml/100 l. Cucumber seeds were sterilized with 1% sodium hypochlorite for 1 min and then rinsed three times with sterile distilled water. The sterilized seeds were added to the prepared suspensions and mixed in a shaker for 30 minutes. Bacteria-coated seeds were transferred on dryer sheets and left to dry in a sterile cabinet. At the end of 24 hours, cucumber seeds coated with bacteria were planted in sterile viols filled with sterile peat (Akbaba & Özaktan, 2018). When the plants reached the 3-leaf stage, they were transferred to pots.

2-Soil drenching

Prepared bacterial suspensions were applied to the roots of cucumber seedlings when the seedlings passed the one-leaf stage by injecting 5 ml of the suspension. When the plants reached the 3-leaf stage, they were transferred to pots.

The experiment was finalized 60 days after *M. incognita* application. Throughout the experiment, plant height was measured weekly, and the number of leaves was recorded. At the end of the experiment, to determine the effectiveness of the treatments on the nematode, the roots of J2 treated cucumber roots were analyzed according to the Zeck (1971) scale. Egg masses in the roots were counted to determine whether the bacterial treatments had a reducing effect on the reproduction of J2s in the roots. Also, J2s in the soil samples taken from the pots were counted and the final population of nematodes was recorded. The numbers of knots in the roots, egg masses and the number of J2 in the soil were analyzed. In addition, to determine the plant growth, at the end of the experiment, after the plants were harvested and the roots were cleaned from the soil, the wet weights of the roots were measured with a sensitive scale. The roots and green parts of the plants were dried in an oven at 80ºC for 48 hours. After drying, the dry weights of the roots and green parts were measured with a sensitive scale.

Data analysis

R statistical software program was used for the analysis of variance (ANOVA) of the values obtained after the experiment was completed, and the comparison of the averages was made according to LSD test at $p \leq 0.05$ level.

Results

The identification of root-knot nematodes was made by using the Method of Preparation of Perineal Samples given by Taylor & Netsher (1974) and developed by Hartman & Sasser (1985). At the end of the experiment, the female root nematodes were obtained from the infected roots. When the preparations from the perineal patterns of the females were analyzed, it was identified that the individuals were belonging to *Meloidogyne incognita* (Kofoid & White, 1919) species.

At the end of the experiment, species identification of the specific rhizobacteria isolates used in the experiment was made. The results of the identification are given in Table 1.

Table 1. Specific rhizobacteria isolates identification

Isolates	Species
KD29	*Pantoea vagans* C (Enterobacterales: Enterobacteriaceae)
KD157	*Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae)
KD238	*Pseudomonas* sp. Migula (Pseudomonadales: Pseudomonadaceae)

Effect of bacterial extracts on J2 immobility and mortality *in vitro* trials

Abbott formula was used to calculate the effect of bacterial isolates on the mortality rate of *Meloidogyne incognita* J2. The results of the experiment are given in Table 2.

Table 2. Reducing effect rate (%) of *in vitro* experiment in the laboratory according to the counts at the end of 96 h

Treatments	Numbers of active nematodes								Percent effect (%)
	24 h		48 h		72 h		96 h		
Control	106.50	a*	91.12	a	85.75	a	76.50	a	-
Sterile Water	105.25	ab	91.12	a	85.50	a	74.00	a	3.26
KD29	97.50	abc	80.87	ab	73.87	ab	55.25	bc	27.77
KD238	94.87	bcd	82.50	ab	63.12	b	50.50	bc	33.98
QST713	92.62	cd	82.75	ab	66.00	b	55.87	b	26.96
KD157	85.12	d	71.00	b	64.12	b	42.25	c	44.77
F	4,739		2,552		4,989		8,552		
p	<0.0001		<0.0001		<0.0001		<0.0001		
df	5, 42		5, 42		5, 42		5, 42		

* Means with the same letter are not statistically different according to LSD test (p≤0.05).

According to these results, control and sterile water treatments had the lowest mortality rate. The highest mortality rate was recorded in *Bacillus thuringiensis* KD157 (44.77%), followed by *Pseodumonas* sp. KD238 (33.98%), *Pantoea vagans* KD29 (27.77%) and *B. subtilis* QST713 (26.96%). These results showed that all bacterial treatments had mortality rates on nematodes significantly higher than the control group.

Effect of rhizobacteria on *Meloidogyne incognita* J2 *in vivo* trials

In order to determine the effectiveness of the bacterial treatments on the amount of root-knot, the roots of the nematode-treated plants were scored according to the Zeck (1971) scale (Table 3).

Compared to the positive control, it was observed that all treatments decreased the amount of root knots. S.D.29 (57.97%) was found to be the most effective treatment to decrease the amount of root knot. This treatment was closely followed by S.D.238 (56.52%) and S.T.QST713 (55.07%). The other treatments had a decreasing effect on the amount of root knot, respectively; S.T.238 (42.03%), S.T.157 (42.03%), S.D.QST713 (40.58%), S.D.157 (40.58%) and S.T.29 (34.78%). As a result of the experiment, it was observed that all bacterial isolates used in the experiment were found to be effective against the root knots caused by the feeding of *Meloidogyne incognita* in cucumber plants.

At the end of the study, the egg masses in the nematode treated plant roots were counted and the effect of bacterial treatments on egg mass production was determined. As a result of the statistical analysis, none of the treatments were in the same group with the positive control. The highest number of egg masses

was found in the positive control (31.09±12.10) and the lowest number of egg masses was found in the S.D.29 (12.18±5.38) treatment. When the bacterial treatments were compared with the positive control, it was observed that all bacterial treatments had a decreasing effect on egg mass production. S.D.29 (60.82%) treatment had the highest decreasing effect on egg mass production followed by S.D.238 (57.31%), S.T.157 (51.17%) and S.D.157 (50.00%) treatments.

Table 3. Effect of rhizobacteria applications against *Meloidogyne incognita* on cucumber

Treatments**	Zeck scale index (X±SD)		Percent effect on root galling	Zeck scale index F (df); p	Egg mass index (X±SD)		Percent effect on egg masses	Egg mass index F (df); p
Positive Control	6.27±1.01	a*	-		31.09±12.10	a*	-	
S.T.29 (N+)	4.09±1.30	b	34.78		18.91±5.56	b	39.18	
S.D.QST.713 (N+)	3.73±2.24	b	40.58		15.91±8.42	bcd	48.83	
S.D.157 (N+)	3.73±1.19	bc	40.58		15.55±7.55	bcd	50.00	
S.T.157 (N+)	3.64±1.43	bc	42.03	F (8, 90)= 7,145; p <0.0001	15.18±4.85	bcd	51.17	F (8, 90)= 6,694; p <0.0001
S.T.238 (N+)	3.64±1.43	bcd	42.03		18.18±4.43	b	41.52	
S.T.QST713 (N+)	2.82±0.60	cde	55.07		16.82±6.01	bc	45.91	
S.D.238 (N+)	2.73±1.49	de	56.52		13.27±6.05	cd	57.31	
S.D.29 (N+)	2.64±0.92	e	57.97		12.18±5.38	d	60.82	

* Means with the same letter are not statistically different according to LSD test (p≤0.05);

** Abbreviations: S.T.: Seed Treatment, S.D.: Soil Drenching.

For all the nematode treated variables, 1500 J2 was applied to the pots as a starting population. At the end of the experiment, 100 g of soil sample was taken from each pot and *M. incognita* J2 in the soil was sampled. Analysis of variance was applied to the J2 numbers determined and LSD test was performed to determine the effect of treatments on nematode population. The results obtained are given in Table 4.

Table 4. Effects of *Meloidogyne incognita* individuals in soil on J2 number (number/100 g) and reproduction rate

Treatments**	Number of Nematodes (X±SD)		F (df); p	Percent effect (%)	RF=Pf/Pi
Positive Control	3967.27±2451.89	ab*		-	2.64
S.T.238 (N+)	4574.55±4323.60	a		-15.33	3.05
S.T.157 (N+)	3359.09±2486.85	abc		24.20	2.24
S.D.157 (N+)	3007.27±4132.67	bc		41.34	2.00
S.T.QST713 (N+)	2327.27±1642.61	cd	F (8, 90)= 2,260; p <0.05	43.17	1.55
S.T.29 (N+)	2254.55±1296.43	cd		49.59	1.50
S.D.29 (N+)	2000.00±2467.39	cd		67.46	1.33
S.D.QST713 (N+)	1290.91±1281.76	de		73.88	0.86
S.D.238 (N+)	1036.36±1130.73	de		77.34	0.69

* Means with the same letter are not statistically different according to LSD test (p≤0.05);

** Abbreviations: S.T.: Seed Treatment, S.D.: Soil Drenching.

When the treatments were compared with the positive control, it was found that all treatments except S.T.238 (-15.33%) influenced the number of *M. incognita* J2 in the soil. S.D.238 (77.34%) was found to be the most effective treatment in decreasing the number of *Meloidogyne incognita* J2 in the soil.

The effects of the treatments on the reproduction rate of the root-knot nematode population were studied. It was determined that S.D.238 (0.69) and S.D.QST713 (0.86) treatments had a reducing effect on the reproduction rate of *M. incognita* J2 in the soil.

Plant height and number of leaves were recorded weekly during the experiment. At the end of the experiment, root growth, root and green parts wet and dry weights were measured to analyze the effect of bacterial treatments on plant growth. However, as a result of the analyses, there was no statistically significant effect of bacterial treatments on the growth and development of cucumber plants.

The effects of the treatments on the reproduction rate of the root-knot nematode population were studied. It was determined that S.D.238 (0.69) and S.D.QST713 (0.86) treatments had a reducing effect on the reproduction rate of *M. incognita* J2 in the soil.

Plant height and number of leaves were recorded weekly during the experiment. At the end of the experiment, root growth, root and green parts wet and dry weights were measured to analyze the effect of bacterial treatments on plant growth. However, as a result of the analyses, there was no statistically significant effect of bacterial treatments on the growth and development of cucumber plants.

Discussion

Soil drenching treatment of *Pantoea vagans* KD29 bacterial isolate was more effective in reducing the amount of root growth compared to seed coating treatment. Soil drenching and seed treatment of this bacterial isolate had 57.97% and 34.78% decreasing effect on the amount of knot on roots, respectively, when compared to the positive control. Mohamedova et al. (2016), in a similar study using *Pantoea agglomerans* isolate against *M. incognita* in eggplant plants, it was reported that it significantly reduced the number of J2 in plants subjected to root dipping and soil drenching treatments, and in the same study, it was reported that the amount of knots on the roots of plants showed a decreasing effect by 32.4% in the seed treatment and 44.6% in the soil drenching treatment compared to the positive control. As a result of the experiment, when the J2 population in the soil was compared with the positive control, *P. vagans* KD29 isolate had an effect of 67.46% in the soil drenching treatment, while this rate was 49.59% in the seed treatment.

However, when the effects of the treatments on the reproduction rate (RF) of the *M. incognita* population were analyzed, it was determined that *P. vagans* KD29 isolate had no effect on the nematode population in two different treatments. Soil drenching treatment of *P. vagans* KD29 isolate reduced the number of egg clusters by 60.82%. While this rate indicated the highest reduction effect among all treatments, the seed treatment of the same isolate had the lowest reduction effect with 39.18%. Although these results were obtained in the *in vivo* trial in the climate chamber, in the *in vitro* trial, when the J2s in the *P. vagans* KD29 solution were counted at the end of 96 hours, the mortality rate was 27.77% compared to the control group. On the other hand, Gowda et al. (2022) researched the activity of *Bacillus subtilis* DTBS 5, *Pantoea agglomerans* and *Bacillus amyloliquefaciens* DSBA 11 isolates against *M. incognita* in an *in vitro* study. In the *in vitro* study, it was observed that the isolates used at 100% concentration were 91.67% effective on J2 death after 96 hours.

When the treatments of *B. thuringiensis* KD157 bacterial isolate were compared with the positive control, seed coating treatment reduced the amount of root growth by 42.03% and soil drenching treatment decreased the amount of root knot by 40.58%. Elsharkawy et al. (2015), in a study conducted with *B. thuringiensis* CR-371 isolate against *M. incognita* in tomato plants, reported that while the root knotting rate of the plants in positive control group was 24.4%, this rate decreased to 5.5% in the roots of the plants treated with *B. thuringiensis* CR-371. In the present study, soil drenching treatment of *B. thuringiensis* KD157 bacterial isolate had a 41.34% reduction effect on the J2 population in the soil compared to the positive control, while this rate was 24.20% in the seed treatment. These results support the study of Khalil & El-Naby (2018), in which the use of *Bacillus thuringiensis* isolate against *M. incognita* in tomato decreased the number of knots in the root by 66.22% to 78.88% and the nematode population in the soil by 70.63% to 80.45%. In the present study, the seed coating treatment of *B. thuringiensis* KD157 isolate had a 51.17% reducing effect on the number of egg clusters, while this rate was 50.00% in the soil drenching treatment. In the greenhouse trial conducted by Khalil et al. (2012) against *M. incognita* on tomato plants, *B. thuringiensis* isolate reduced the J2 population in the soil by 80.5%, while *B. thuringiensis* prevented egg mass production by 74.9%.

In a similar study conducted by Dawar et al. (2008), *B. thuringiensis* (Bt-10) was tested against *M. javanica* by seed coating and soil drenching on mash bean and cowpea. It was reported that there was no significant difference between the application methods and both methods significantly reduced nematode damage in both plant varieties. Choi et al. (2020), in an *in vivo* trial to research the efficacy of *B. thuringiensis* KYC isolate against *M. incognita*, it was reported that the treatment significantly decreased the egg mass production in tomato plants with fertilizer alone. While these results were obtained in the *in vivo* trial in the climate chamber, when the J2s in the *B. thuringiensis* KD157 bacterial solution used in the *in vitro* trial in the laboratory were counted at the end of 96 hours, the mortality rate was 42.25% compared to the control group. This isolate was the bacterial isolate with the highest lethal effect against *M. incognita* in the *in vitro* trial compared to other treatments. Dawar et al. (2008) conducted an *in vitro* study with *B. thuringiensis* (Bt-10) isolate and found that the isolate eliminated 50% of *M. javanica* J2 survival and egg hatching. In a similar *in vitro* study conducted by Oliveira et al. (2007), it was reported that *B. thuringiensis* isolates reduced the number of J2 of *M. exigua*.

Soil drenching treatment of *Pseudomonas* sp. KD238 bacterial isolate reduced the amount of root growth by 56.52% compared to the positive control, while seed coating treatment reduced the amount of root growth by 42.03%. These results support the results of Kaşkavalcı et al. (2006), who found that seed treatment and seed treatment + soil drenching treatment of *Pseudomonas fluorescens* Pat1 strain reduced the root growth of *M. incognita* by 44% and 39%, respectively, in the climate chamber *in vivo* trial against *M. incognita* in cucumber plants. *Pseudomanas* sp. KD238 isolate had the highest effect on the decrease of *M. incognita* J2 number in the soil because of soil wetting application. As a result of the experiment, when the J2 population in the soil was compared with the positive control, the soil wetting treatment had a 77.34% reduction effect, while this rate was found to be -15.33% in the seed treatment.

When the effects of the treatments on the reproduction rate of the J2 population were examined, it was determined that the soil wetting treatment of *Pseudomanas* sp. KD238 isolate had the highest reducing effect on the J2 reproduction rate in the soil with a value of 0.69. This rate was lower than the reproduction rate of 0.86 of the commercial preparation QST713, which was the control group. These results support the results of Ashoub & Amara (2010), who reported that *P. fluorescens* RR isolate was highly effective in suppressing *M. incognita in vitro* and *in vivo* studies. In the soil drenching treatment of *Pseudomanas* sp. KD238 isolate, a 57.31% decreasing effect on the number of egg masses was seen, while this rate was 41.52% in the seed treatment. When J2s in *Pseudomanas* sp. KD238 bacterial solution were counted at the end of 96 hours in the *in vitro* experiment in the laboratory, it was determined that the mortality rate was 33.98% compared to the control group.

The results of the present study were similar to those of *in vitro* and *in vivo* trials established by Singh et al. (2021) to study the biocontrol potential of *P. fluorescens* against *M. incognita*. It was reported that *P. fluorescens* inhibited *M. incognita* egg hatching by 75% and caused 100% J2 mortality. In the same study, in the *in vivo* trial, *P. fluorecens* isolate was found to reduce egg mass, egg production, number and size of eggs when applied at a dose of 10^9 (CFU/ml) against *M. incognita*. In a similar study reported by Abd-El-Khair et al. (2019), *P. fluorescens* (Pf1, Pf2) isolates were applied separately to cowpea plants in pots and inhibited the reproduction of *M. incognita* population by 69.8% and 62.3%, respectively. In a similar study by Singh et al. (2021), *P. fluorescens* isolate applied to tomato plants increased the weight of plant roots and shoots. However, in the present study, there was no change in the weight of root-green parts of cucumber plants treated with *Pseudomonas* sp. KD238 isolate compared to the negative control. In a similar study conducted by Almaghrabi et al. (2013) against *M. incognita* in tomato plants, it was reported that plant dry weight and plant height increased, while the amount of knot in the root, egg mass and the number of J2 in the soil decreased in the variables treated with *P. fluorescens* isolate.

When the data obtained were analyzed, it was shown that bacterial isolates did not have a significant effect on plant growth, but seed treatment of bacteria had a slight effect on root growth, plant height and leaf number compared to the negative control. It was found that all bacterial treatments had a decreasing effect on the amount of root-knot infections on the roots of cucumber plants. Soil drenching treatment (57.97%) of *P. vagans* KD29 isolate was the most successful treatment on the decrease in the amount of root knots. In addition, all the bacterial treatments significantly decreased the egg mass production on the roots compared to the positive control. Soil drenching treatments of *P. vagans* KD29 (60.82%) and *Pseudomonas* sp. KD238 isolates (57.31%) were found to have the highest decreasing effect on egg mass formation on roots.

At the end of the experiment, when the bacterial treatments were compared with the positive control, the soil wetting treatment of *Pseudomonas* sp. KD238 isolate (77.34%) was found to be the most effective treatment in reducing the number of *M. incognita* J2 in the soil. It was found that soil drenching treatments of bacterial isolates were more effective in decreasing the egg mass production on the roots and J2 population in the soil than seed treatment. Soil drench treatments of the specific bacterial isolates *P. vagans* KD29 and *Pseudomonas* sp. KD238 were found to be more successful in decreasing the amount of knots and the number of egg masses in the roots than the soil drenching treatment of *B. subtilis* QST713 commercial preparation used as a control. Soil drench treatment of *Pseudomonas* sp. KD238 isolate was more successful in decreasing the reproduction rate of *M. incognita* population in soil than the soil drench treatment of *B. subtilis* QST713 commercial preparation.

Pseudomonas sp. KD238 and *P. vagans* KD29 isolates were found to have 33.98% and 27.77% lethal effect on J2s, respectively, in the *in vitro* test. However, these bacteria are thought to promote induced systemic resistance (ISR), especially considering the decrease in the J2 population in the soil, the amount of root knot and the decrease in the number of egg masses. *Pseudomonas* sp. KD238 and *P. vagans* KD29 are important to be studied to understand the mechanism of action of bacterial isolates.

It is thought that the specific bacterial isolates used in this study may be an alternative to the use of nematicides in the control of root-knot nematode, which causes significant damage to cucumber plants. However, it is thought that further studies on the use of these bacterial isolates on cucumber plants in greenhouses and open fields will contribute more to this issue.

Acknowledgments

We would like to thank Prof. Dr. Hatice Özaktan for providing the bacterial isolates used in the study from her own stock and sharing her valuable ideas and opinions. We would like to thank Dr. Utku Şanver for his help with the statistical analysis of the experimental data; Esmeray Ayhan Cafarlı for her help with the collection of the nematode population and, Barış Çipli for guiding with his knowledge and experience. We would also like to extent my heartfelt thanks to Esra Partal, Ayten Özay, İrem Dağ, Mehmet Emin Kurter in the Nematology laboratory and Gizem Toyğar in the Bacteriology laboratory for their support in conducting this study.

References

Abd-El-Khair, H., W. M. A. El-Nagdi, M. M. A. Youssef, M. M. M. Abd-Elgawad & M. G. Dawood, 2019. Protective effect of *Bacillus subtilis, B. pumilus*, and *Pseudomonas fluorescens* isolates against root knot nematode *Meloidogyne incognita* on cowpea. Bulletin of the National Research Centre, 43 (1): 1-7.

Akbaba, M. & H. Özaktan, 2018. Biocontrol of angular leaf spot disease and colonization of cucumber (*Cucumis sativus* L.) by endophytic bacteria. Egyptian Journal of Biological Pest Control, 28 (1): 1-10.

Almaghrabi, O.A., S. I. Massoud & T. S. Abdelmoneim, 2013. Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi Journal of Biological Sciences, 20 (1): 57-61.

Anonymous, 2008. Zirai Mücadele Teknik Talimatları Cilt 6. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü, Ankara, 286 s (in Turkish).

Ashoub, A. H. & M. T. Amara, 2010. Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. Journal of American Science, 6 (10): 321-328.

Choi, T. G., C. E. H. Maung, D. R. Lee, A. B. Henry, Y. S. Lee & K. Y. Kim, 2020. Role of bacterial antagonists of fungal pathogens, *Bacillus thuringiensis* KYC and *Bacillus velezensis* CE 100 in control of root-knot nematode, *Meloidogyne incognita* and subsequent growth promotion of tomato. Biocontrol Science and Technology, 30 (7): 685-700.

Dawar, S., M. Tariq & M. Zaki, 2008. Application of *Bacillus* species in control of *Meloidogyne javanica* (Treub) Chitwood on cowpea and mash bean. Pakistan Journal of Botany, 40 (1): 439-444.

Echeverrigaray, S., J. Zacaria & R. Beltrão, 2010. Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. Phytopathology, 100: 199-203.

Elsharkawy, M. M., M. Nakatani, M. Nishimura, T. Arakawa, M. Shimizu & M. Hyakumachi, 2015. Control of tomato bacterial wilt and root-knot diseases by *Bacillus thuringiensis* CR-371 and *Streptomyces avermectinius* NBRC14893. Acta Agriculturae Scandinavica Section B: Soil and Plant Science, 65 (6): 575-580.

FAOSTAT, 2021. Value of agricultural production. (Web page: https://www.fao.org/faostat/en/#data/QV) (Date accessed: 12.06.2023).

Gowda, A. P. A., B. Pankaj, D. Singh, A. K. Singh & R. Sowmya, 2022. Nematicidal potential of plant growth-promoting rhizobacteria against *Meloidogyne incognita* infesting tomato under protected cultivation. Egyptian Journal of Biological Pest Control, 32 (1): 145 (1-13).

Kaşkavalcı, G., H. Özaktan, A. Hatipoğlu & A. Uslu, 2006. Preliminary investigations on suppression of *Meloidogyne incognita* (Kofoid & White) Chitwood (Nematoda: Heteroderidae) by antagonistic rhizobacteria. Türkiye Entomoloji Dergisi, 30 (3): 173-182.

Khalil, M. S. & S. S. El-Naby, 2018. The integration efficacy of formulated abamectin, *Bacillus thuringiensis* and *Bacillus subtilis* for managing *Meloidogyne incognita* (Kofoid & White) Chitwood on tomatoes. Journal of Biopesticides, 11 (2): 146-153.

Khalil, M. S., A. Kenawy, M. A. Gohrab & E. E. Mohammed, 2012. Impact of microbial agents on *Meloidogyne incognita* management and morphogenesis of tomato. Journal of Biopesticides, 5 (1): 28-35.

Mohamedova, M., D. Draganova, I. Valcheva & M. Naydenov, 2016. Effects of Rhizobacteria on *Meloidogyne javanica* infection on eggplants. African Journal of Agricultural Research, 11 (41): 4141-4146.

Netscher, C. & R. A. Sikora, 1990. "Nematode Parasites on Vegetables, 231-283". In: Plant Parasitic Nematodes in Suptropical and Tropical Agriculture (Eds. M. Luc, R. A. Sikora & J. Bridge) CAB International, UK, 629 pp.

Oka, Y., 2020. From old-generation to next-generation nematicides. Agronomy, 10 (9):1387.

Oliveira, D. F., V. P. Campos, D. R. Amaral, A. S. Nunes, J. A. Pantaleao & D. A. Costa, 2007. selection of Rhizobacteria able to produce metabolites active against *Meloidogyne exigua*. European Journal of Plant Pathology, 119 (4): 477-479.

Paul, D. & H. Lade, 2014. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. Agronomy for Sustainable Development, 34 (4): 737-752.

Singh, S., R. Balodi, P. N. Meena & S. Singhal, 2021. Biocontrol activity of *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia solani*. Indian Phytopathology, 74 (3): 703-714.

Tian, B., J. Yang & K.Q. Zhang, 2007. Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. FEMS Microbiology Ecology, 61 (2): 197-213.

TÜİK, 2022. Bitkisel üretim istatistikleri. (Web page: https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2022-45504) (Date accessed: 12 June 2023) (in Turkish).

Zeck, W. M., 1971. A Rating Scheme for Field Evaluation of Root-knot Nematode Infestation. Pflanzenschutz Nachrichten, Bayer. Published by Farbenfabriken Ag. Leverkusen, 10 (1): 361 pp.