

**Original article (Orijinal araştırma)**

**Allelopathic effect of *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae) and *Rhododendron ponticum* L. (Ericales: Ericaceae) essential oils and extracts on *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae)<sup>1</sup>**

*Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae) ve *Rhododendron ponticum* L. (Ericales: Ericaceae) bitkilerinin uçucu yağ ve ekstraktlarının *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) üzerinde allelopatik etkisi

Taylan ÇAKMAK<sup>2\*</sup> 

Zübeyde Filiz ARSLAN<sup>3</sup> 

Salih YURTTAŞ<sup>3</sup> 

Murat Emin TAŞ<sup>2</sup> 

**Abstract**

In this study, the nematicidal activity of essential oils and extracts of *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae) and *Rhododendron ponticum* L. (Ericales: Ericaceae) against the second-stage juveniles (J2s) of *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) were evaluated in Petri dish and pot experiments. The essential oils and extracts were applied directly to J2s at concentrations of 5 and 10% (w/v). The mortality of J2s of *M. incognita* was recorded 24, 48, and 72 hours after treatments. The results showed that a 100% mortality rate was obtained from both concentrations (5 and 10%) of *O. syriacum* var. *bevanii* essential oil application. Moreover, the pot experiment showed that *O. syriacum* var. *bevanii* essential oil (5%) (w/v) and *O. syriacum* var. *bevanii* extract (10%) was more effective against the J2s of *M. incognita* on tomato plants. Results were promising in terms of testing the effects of essential oil and extracts obtained from the determined plants in laboratory conditions against *M. incognita*.

**Keywords:** Antagonistic plants, biopesticides, Root-knot nematodes, susceptibility

**Öz**

Bu çalışmada, dağ kekiği, *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae), ve ormangülü, *Rhododendron ponticum* L. (Ericales: Ericaceae) bitkilerinden elde edilen uçucu yağ ve ekstraktlarının Kök-ur nematodu, *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) ikinci dönem larvaları üzerinde allelopatik etkisini, petri ve saksı denemelerinde belirlemek amaçlanmıştır. Laboratuvar denemelerinde iki farklı dozda dağ kekiği yağı (%5-10), dağ kekiği ekstraktı (%5-10), ormangülü ekstraktı (%5-10) ve bu iki bitkinin karışım ekstraktının *M. incognita* larvaları üzerine doğrudan uygulanması sonrasında 24, 48 ve 72 saat aralıklarla nematodların ölüm oranları hesaplanmıştır. Saksı denemelerinde ise dağ kekiği yağı (%5-10), dağ kekiği ekstraktı (%5-10), ormangülü ekstraktı ve dağ kekiği + orman gülü ekstraktı (%5-10) uygulamalarının bitki boyuna, köklerde urlanma seviyeleri ve yumurta paket sayıları üzerine etkisi değerlendirilmiştir. Çalışmalar sonucunda, laboratuvar denemesinde kekik yağının her iki dozunda da (%5 ve %10) %100 ölüm oranı tespit edilmiştir. Saksı denemelerinde ise %5'lik kekik yağı ve %10'luk kekik ekstraktı diğer uygulamalara göre daha etkili bulunmuştur. Bu çalışma ile dağ kekiği ve ormangülü yağ ve ekstraktlarının *M. incognita* ikinci dönem larvalarına biyolojik etkisi açısından ümitvar sonuçlar elde edilmiştir.

**Anahtar sözcükler** Antagonistik bitkiler, biyopestisitler, Kök-ur nematodları, duyarlılık

<sup>1</sup> This study was supported by Düzce University, Scientific Research Unit, Agricultural Biotechnology, Türkiye.

<sup>2</sup> Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, 81620, Düzce, Türkiye

<sup>3</sup> Düzce University, Faculty of Agriculture, Department of Plant Protection, 81620, Düzce, Türkiye

\* Corresponding author (Sorumlu yazar) e-mail: [cakmaktaylan@gmail.com](mailto:cakmaktaylan@gmail.com)

Received (Alınış): 30.05.2023

Accepted (Kabul edilmiş): 11.11.2023

Published Online (Çevrimiçi Yayın Tarihi): 22.11.2023

## Introduction

The need for safe food resources, which has increased due to the rapid increase in the world population in recent years, is directly related to the sustainability principle of agricultural production. Thus, countries are searching for innovative solutions by reviewing their agricultural policies. Root-knot nematodes (RKNs), *Meloidogyne* spp. (Rhabditida: Meloidogynidae) are organisms that live as obligate parasites on plants, with a wide distribution and host range worldwide especially in tropical and semi-tropical climate zones causing harmful results in agricultural production areas (Whitehead, 1997). Due to their complex biology, the development of plant roots slows down or is irregular and finally these plants cannot develop healthily and become stunted. Plants with extensive RKNs infestation can dry out completely (Thorne, 1961). It is common that synthetic pesticides are frequently used in the control of RKNs. This situation poses a threat to the environment and human health. Resistance develops in nematodes against pesticides and at the same time, the pesticide residues accumulate on crops and move to underground freshwater in the long term (Moens et al., 2009). For such reasons, the importance given to alternative methods that do not harm nature and can be used instead of common chemical applications has increased in recent years against RKNs. Plants are natural sources containing many active substances within their rich structure, and more than two thousand plants have been shown to have the potential to be used as bio insecticides so far (Ahmed & Grainge, 1988; Prakash & Rao, 1996; Öncüer, 2000). Furthermore, allelochemicals which are produced by plants are secondary metabolites and have an important place since they have direct or indirect effects on inducing pests and harmful organisms (Gürsoy et al., 2013). To date, there are at least 120 allelochemicals according to Fahey et al. (2001), which are known to have antagonistic interactions with bacteria, nematodes, fungi, and herbivores.

The aim of this study was to assess the effectiveness of thyme, *Origanum syriacum* var. *bevanii* (Holmes) (Lamiales: Lamiaceae) essential oil and extracts, and rhododendron, *Rhododendron ponticum* L. (Ericales: Ericaceae) extracts in vitro conditions against second-stage infective juveniles of *Meloidogyne incognita* (Kofoid & White, 1919) (Rhabditida: Meloidogynidae) both in laboratory and pot experiments.

## Materials and Methods

### Identification and obtaining nematodes pure culture

Root-knot nematodes were obtained from a greenhouse infested RKNs. Samples were collected at 04/07/2022 in Antalya Serik (36°55'34"N, 31°6'17"W), where tomato plants were being cultivated. Once the infested tomato plants were brought to the laboratory, egg masses were collected from infected tomato roots using forceps and were put into incubation for the hatching of second-stage juveniles (J2s). After 48 hours in a modified Baermann funnel, (using 8 cm high, 10-12 cm wide Petri dishes with a sieve inside), the emerging J2s were obtained to be used for plant infestation, with the aim of establishing a pure nematode culture. This process was repeated for 10 days during which the larvae continued to emerge from the egg masses. Initial nematodes were obtained and counted. Ten tomato plants were placed in sterilized sand in a climatic chamber set at 25±1°C, 65±5% relative humidity, and a 16:8 photoperiod, and were infested with 1000 J2s for having a pure culture of *M. incognita*. Pure *M. incognita* populations were obtained after 60 days of the plantation. Nematode-infested roots were washed in water in the laboratory and these roots were cut into 1-3 cm and observed under a light microscope. Female nematodes were once more separated and incubated using the modified Baermann funnel technique to allow J2s emergence over a period of ten days. In order to obtain nematode larvae from RKN-infested tomato plant roots. The sieve on which the egg masses were placed on the filter paper was placed in the Petri dish and was filled with sterile water, and the J2s were allowed to hatch and sink to the bottom in the water, and after 48 hours, after J2s emerged from egg masses, the water in the Petri dish was transferred to the 50 ml tubes. This process was repeated for 10 days during which the J2s continued to emerge from the egg masses. Collected J2s of *M. incognita* were left for six hours to sink to the bottom of the tube. After six hours, the

water was diluted to 15 ml. The collected nematodes were stored in the refrigerator at +4°C. Nematode counts in 1 ml of water was made to calculate the number of larvae when the nematodes were to be used in the experiments. The nematode count was repeated three times. The average number of nematodes was calculated by multiplying the amount of water by the average number of larvae. For the Petri dish experiment, 400 *M. incognita* J2s were used in each application.

## Laboratory experiment

### Obtaining plant essential oils and extracts

In the study, the essential oils and extracts of Thyme (*O. syriacum* var. *bevanii*) and Common rhododendron (*R. ponticum* L.) were applied at two concentrations (%5-10) (w/v). The Thyme and Rhododendron plants were collected from the natural areas of Antalya province, Serik district (37.128695, 30.961959) and morphological identification was made in the Herbology Laboratory of Düzce University. Different parts of the tested plants were taken to prepare their extracts. The chosen parts were stem parts, flowers, and leaves of thyme. These plant origins were washed with distilled water to remove any dust and air-dried in direct sunlight. The dried plant materials were powdered and passed through a 60-mesh sieve. Samples of plant powders were homogenized with a laboratory blender used at 30 g from each plant material in one liter of distilled water for 10 min., and then left in dark glass bottles for 72 hr for tissue disintegration. The extracts were filtered with a Whatman filter paper to get the clear extract. The obtained extracts were dissolved as Thyme and Rhododendron at a rate of 5% and 10% (w/v) and stored in the refrigerator at +4°C. Nematicidal activities of separate and combined applications of Thyme (*O. syriacum* var. *bevanii*) and Common rhododendron (*R. ponticum* L.) were tested on J2s of *M. incognita* as follows: OSEO: *O. syriacum* var. *bevanii* essential oil (%5-10) (w/v), OSE: *O. syriacum* var. *bevanii* extract (%5-10) (w/v) RPE: *R. ponticum* extract (%5-10) (w/v), OSE+RPE: *O. syriacum* var. *bevanii* and *R. ponticum* extract (%5-10) (w/v). This study was conducted in Düzce University, Herbology, and Nematology Laboratories in 2022 and 2023.

The trials were established according to the Random Plots Trial Design with 10 characters (8 treatments + 2 controls), with 3 replications. Plant essential oils and prepared plant extracts were adjusted as 10 ml of solution added to 400 *M. incognita* J2s in 1 ml of water placed in glass Petri dishes at room temperature (Oka et al., 2000). For control trials, only pure water was included into Petri dishes with J2s. The laboratory experiment was repeated twice in 2022 and 2023 at Düzce University, Faculty of Agriculture, Department of Plant Protection Laboratory.






### Pot experiment

The *M. incognita* J2s used in bioassays and field trials were obtained from a laboratory-reared *M. incognita* inoculum originally sourced from an infected greenhouse (04/07/2021) in Serik, Antalya, Türkiye (36°55'34"N, 31°6'17"W). The colony was purified and maintained in Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology at 6±1°C, 60±80% relative humidity. The pot experiment was repeated twice in 2022 and 2023 at Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology Laboratory under controlled climatic conditions in the laboratory (at 25±1°C, 60±80% relative humidity and a photoperiod of 16:8 h light: dark). Essential oil-sand mixture containing 70% sand and 30% potting essential oil was prepared and sterilized in an autoclave at 121°C for 90 minutes.

For each application, 10 tomato plants were grown in 1-liter pots. Four-week-old BT 236 tomato plants were planted in a controlled climatic condition. Afterwards, each pot was inoculated homogeneously with 1000 *M. incognita* J2s in 30ml of distilled water, at four plots with a distance of 3-4 cm from each other near the root stem. Three replicates were applied for each experiment. After *M. incognita* inoculation to tomato plants, in order to assess the efficiency on the control of *M. incognita*, essential oil, extracts at different concentrations (OSEO: *O. syriacum* var. *bevanii* essential oil (%5-10) (w/v), OSE: *O. syriacum* var. *bevanii* extract (%5-10) (w/v) RPE:

*R. ponticum* extract (%5-10) (w/v), OSE+RPE: *O. syriacum* var. *bevanii* and *R. ponticum* extract (%5-10) (w/v) were applied to the pots with a volume of 30 ml of solution on the same day and compared with two control (infected and uninfected) applications. The plants were kept in climate chambers for 10 weeks at a constant temperature of  $27\pm 3^{\circ}\text{C}$ , 70% humidity and 16 hours of light per day. The plants were irrigated on a daily basis. Tomato plants were removed from the pots 10 weeks ( $70\pm 3$  days) after the application of *M. incognita* and plant essential oil and extracts. The roots of the plants were thoroughly washed with water and essential oil debris were removed gently. Egg mass counts were made under a stereo microscope with the aid of a fine needle. Then, the root-knot scale and plant height of each plant were determined according to Feldemesser & Feder (1955), Zeck (1971) and Taylor & Sasser (1978) (Table 1).

Table 1. The infection scale of *Meloidogyne incognita* in the root system (Feldemesser & Feder, 1955; Zeck, 1971)

Root development categories	0	1	2	3	4
Root side section profile					
root development index	No galling	Slightly infected	Reasonably infected	Intensely infected	Extremely infected
gall number index (%)	0	0-25	25-50	50-75	75-100

### Counting and evaluation

After the laboratory experiments, live nematodes were counted with the help of an Olympos® light microscope at 24-48-72 hours after the application.

### Data analysis

The data were statistically evaluated using the JMP 11 package program of the SAS Institute (SAS, 2013). The results from the laboratory experiments (*M. incognita* death/viability ratios) were subjected to a one-way ANOVA with mean percentages of death at 24-48-72 hours post application. The square root transformation was applied to the calculated median values related to the root-knot index values and the original data were given in tables. Because of the variances were homogeneous, the data obtained from two years were subjected to the analysis of variance and "LSD Multiple Comparison Test" was used for comparison of the means at a 0.05 significance level.

## Results and Discussion

### Laboratory experiment

As a result of the morphometric and morphological analysis of the species identification, the nematode species was determined as *M. incognita*. In the evaluations made at the 24-hour post-application counts of the laboratory trials, the minimum number of alive *M. incognita* J2s was determined in 10% (w/v) application of *O. syriacum* var. *bevanii* essential oil (Table 2).

Based on the two-year average values of laboratory experiment, it was determined that the J2s mortality rate of *M. incognita* was highest with a 10% concentration of *O. syriacum* var. *bevanii* essential oil (i) application, followed by 5% (w/v) *O. syriacum* var. *bevanii* essential oil (ii), 10% (w/v) *O. syriacum* var. *bevanii* extract (iii), 5% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (iv), 10% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (v), 5% (w/v) *O. syriacum* var. *bevanii* extract (vi), 10% (w/v) *R. ponticum* extract (vii), 5% (w/v) *R. ponticum* extract (viii). In the 24-hour count evaluations, the highest number of live larvae was determined in 5% *R. ponticum* extract after the control.

Table 2. Effect of treatments on *Meloidogyne incognita* J2s at laboratory condition (2022-2023 mean)\*

Treatments**	% Mortality**		
	24 h	48 h	72 h
OSEO 5%	100.0±0.1 a	100.0±0.0 a	100.0±0.0 a
OSEO 10%	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a
OSE 5%	66.9±2.8 a	70.1±3.8 b	71.5±6.1 cd
OSE 10%	95.01±1.9 b	99.8±0.1 a	99.7±0.1 a
RPE 5%	25.4±9.2 d	39.4±10.5 d	51.6±9.0 e
RPE 10%	36.4±11.7 c	54.2±1.5 c	64.8±9.3 d
OSE 5% + RPE 5%	95.1±1.6 a	96.0±2.1 a	77.9±8.4 bc
OSE 10% + RPE 10%	72.5±4.4 b	76.1±7.1 b	84.7±2.6 b
F	73,3003	60,9626	50,0427
P	<,0001	<,0001	<,0001
DF Total	23	23	23
DF Error	14	14	14

\* Means separation within columns using LSD comparison test at  $\alpha=0.05$ . Data given  $\pm$  are STDEV (standard deviation);

\*\* Abbreviations: OSEO, *O. syriacum* var. *bevanii* essential oil; OSE, *O. syriacum* var. *bevanii* extract; RPE, *R. ponticum* extract.

In the evaluations made at the 48-hour counts of the laboratory experiment, the minimum number of alive J2s among the applications was *O. syriacum* var. *bevanii* essential oil at 10% (w/v) and 5% (w/v) *O. syriacum* var. *bevanii* essential oil with 100% mortality. At the trial counts of 48-hours post application, the highest efficacy was determined as follows: 10% (w/v) *O. syriacum* var. *bevanii* essential oil (i), 5% (w/v) *O. syriacum* var. *bevanii* essential oil (ii), 10% (w/v) *O. syriacum* var. *bevanii* extract (iii), 5% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (iv), 10% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (v), 5% (w/v) *O. syriacum* var. *bevanii* extract (vi), 10% (w/v) *R. ponticum* extract (vii) and 5% (w/v) *R. ponticum* extract (viii).

In the 48-hour counting evaluations, the highest number of alive J2s was determined in the 5% (w/v) *R. ponticum* extract after the control. In the evaluations made at the 72-hour post application, the minimum number of alive J2s among all applications was 10% (w/v) *O. syriacum* var. *bevanii* essential oil and 5% (w/v) *O. syriacum* var. *bevanii* essential oil with 100% mortality. Mortality levels were detected respectively as follows; 10% (w/v) *O. syriacum* var. *bevanii* essential oil (i), 5% (w/v) *O. syriacum* var. *bevanii* essential oil (ii), 10% (w/v) *O. syriacum* var. *bevanii* extract (iii), 10% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (iv), 5% (w/v) *O. syriacum* var. *bevanii* + *R. ponticum* extract (v), 5% (w/v) *O. syriacum* var. *bevanii* extract (vi), 10% (w/v) *R. ponticum* extract (vii) and 5% (w/v) *R. ponticum* extract (viii). In the 72-hour counting evaluations, the highest number of alive J2s after the control was determined in the 5% (w/v) *R. ponticum* extract.

### Pot experiment

Three characters were evaluated for each plant which were determined as follows: (i) Plant height (cm), (ii) egg package index (number of *M. incognita* eggs found on each plant), and (iii) gall number index which refers to the severity level of the infection and gall occurrence of each tomato plant. In the evaluations of plant height, egg package index, and gall number index of the pot experiment, *O. syriacum* var. *bevanii* essential oil (OSEO) (5%) and (OSEO) (10%) were statistically found to be significantly different than other applications (OSEO 5%: Plant height: 56.6±2.3 cm Egg package index: 0.51±0.56; Gall number index: 0.08±0.14), (OSEO 10%: Plant height: 53.61±4.2; Egg package index: 0.43±0.18; Gall number index: 0.08±0.14) Also, *O. syriacum* var. *bevanii* extract (OSE) of 10% was found to be close to uninfected control (OSE 10%: Plant height: 56.78±1.2; Egg package index: 2.0±0.59; Gall number index: 0.33±0.29). These

three applications of *O. syriacum* var. *bevanii* essential oil and extract were found to be the most effective applications against *M. incognita* J2s on tomato plants followed by: OSE 5%: Plant height: 54.38±1.0; Egg package index: 1.8±0.78; Gall number index: 0.33±0.29; RPE 10%: Plant height: 51.0±2.5; Egg package index: 3.3±1.03; Gall number index: 1±0.0; OSE 5% + RPE 5%: Plant height: 50.2±3.7; Egg package index: 3.65±0.95; Gall number index: 0.58±0.14; RPE 5%: Plant height: 47.31±6.9; Egg package index: 3.9±0.83b; Gall number index: 0.75±0.43; OSE 10% + RPE 10%: Plant height: 46.9±1.3; Egg package index: 3.3±0.25; Gall number index: 0.83±0.29) (Table 3-4).

Table 3. Plant height (cm), egg package index and gall number index ratio (2022-2023 mean) (Feldmesser & Feder, 1955; Zeck, 1971)

Treatments**	Plant height (cm)	Egg package index	Gall number index
OSEO 5%	56.60±2.30 ab	0.51±0.56 cd	0.08±0.14 de
OSE 5%	54.38±1.00 bc	1.80±0.78 bc	0.33±0.29 cde
RPE 5%	47.31±6.90 de	3.90±0.83 b	0.75±0.43 abc
OSE 5% + RPE 5%	50.20±3.70 cd	3.65±0.95 b	0.58±0.14 bcd
OSEO 10%	53.61±4.20 bc	0.43±0.18 cd	0.08±0.14 de
OSE 10%	56.78±1.20 ab	2.0±0.59 b	0.33±0.29 cde
RPE 10%	51.00±2.50 cd	3.30±1.03 b	1.00±0.00 ab
OSE 10% + RPE 10%	46.90±1.30 de	3.30±0.25 b	0.83±0.29 ab
Control (+)	43.30±2.00 e	6.18±1.06 a	1.25±0.25 a
Control (-)	59.20±1.30 a	0.00±0.00 d	0.00±0.00 e
F	8,3932	2,9131	2,9131
P	<,0001	0,0020	0,0020
DF Total	59	59	59
DF Error	38	38	38

\* Means separation within columns using LSD comparison test at  $\alpha=0.05$ , Data given  $\pm$  are STDEV (standard deviation);

\*\* Abbreviations: OSEO, *O. syriacum* var. *bevanii* essential oil; OSE, *O. syriacum* var. *bevanii* extract; RPE, *R. ponticum* extract.

Table 4. Plant height (cm), egg package index and gall number index (2022 and 2023) (Feldmesser & Feder, 1955; Zeck, 1971)

Treatments**	Plant height (cm)		Egg package index		Gall number index	
	2022	2023	2022	2023	2022	2023
OSEO 5%	59.97 ab*	53.23 a*	0.47 cd*	0.57 ef*	0.17 c*	0.00 e*
OSE 5%	58.90 ab*	49.87 abc*	1.97 bcd*	1.63 bc	0.33 bc*	0.33 cde*
RPE 5%	48.93 cd	45.70 cde	3.47 b	4.37 ab	0.67 abc	0.83 abc
OSE 5% + RPE 5%	55.63 bc	44.90 cde	4.03 b	3.27 bc	0.67 abc	0.5 de
OSEO 10%	55.50 bc	51.60 ab*	0.43 cd*	0.43 def*	0.00 c*	0.17 de*
OSE 10%	61.00 ab*	52.57 ab	2.90 b	1.27 cde	0.50 bc*	0.17 de*
RPE 10%	54.30 bc	47.83 bcd	2.50 bc	4.10 abc	1.00 ab	1.00 ab
OSE 10% + RPE 10%	51.00 cd	42.80 de	3.87 b	2.80 abc	1.00 ab	0.67 abcd
Control (+)	44.67 d	41.93 e	6.40 a	5.97 a	1.33 a	1.17 a
Control (-)	63.53 a	55.00 a	0.00 d	0.00 f	0.00 c	0.00 e
F	5,1474	7,1204	5,4811	7,3045	3,0949	4,4533
P	0,0011	0,0001	0,0008	0,0001	0,0164	0,0026
DF Total	29	29	29	29	29	29
DF Error	19	19	19	19	19	19

\* Means separation within columns using LSD comparison test at  $\alpha=0.05$ ;

\*\* Abbreviations: OSEO, *O. syriacum* var. *bevanii* essential oil; OSE, *O. syriacum* var. *bevanii* extract; RPE, *R. ponticum* extract.

The unconscious use of pesticides and non-compliance with the rules in practice destroy the ecological balance. At the same time, intensive use of pesticides causes the extinction of many beneficial species. RKNs are among the most important plant pests in agriculture worldwide.

Chemical origin plant protection products are used for controlling RKNs, but studies on alternative control methods have gained momentum in recent years due to the negative effects of these nematicides, most of which have systemic effects, on the environment and human health. In this process, it has been revealed that biochemical compounds obtained from plants can be used in the control of RKNs.

In this context, nematicides of plant origin can be investigated more intensively and their application areas can be expanded and they have a high potential to be used as a general control agent in suppressing the population of not only RKNs but also other plant parasitic nematodes in the essential oil. Thanks to the data obtained from these studies, an environmentally friendly control strategy against nematodes can be followed.

As a result of the laboratory trials conducted in this study, *O. syriacum* var. *bevanii* essential oil and *O. syriacum* var. *bevanii* extracts were the most effective applications with a 100% mortality rate in both concentrations (5-10%) (w/v) applied to the J2s *M. incognita* larvae. Some thyme species (*T. capitatus*, *O. vulgare*, *O. dictamnus*, *O. majorana*) have antagonistic relation with *Fusarium solani*, along with essential oils of lavender, rosemary, sage, and watermelon (Daferera et al., 2003). The extract obtained from the dried mint plant showed a significant nematicide effect against *M. incognita* J2s (Caboni et al., 2013).

With respect to the potential of using some plant extracts in the control of RKNs, Walker & Melin (1996) found that when the extracts of some mint cultivars were used, this caused significant reductions in root gall formation despite high nematode infestation, and that these mint cultivars were host to *M. incognita* and *M. arenaria*. In addition, they found that after growing the thyme plant in nematode-infested essential oil for a period of 12 weeks, the rate of galling was reduced by 90% in sensitive tomato roots planted, compared to tomatoes grown in the control group.

When the results from the previous studies and the current study are evaluated together, it is thought that the nematode population can be suppressed as a result of the thyme plant being grown in nematode-infested areas before the cultivated plant to be produced and mixed with the essential oil as green manure. The plant essential oils and extracts, whose effectiveness was evaluated in this study, are widely distributed in our country and can be easily obtained.

This study has yielded promising results with the potential to be an alternative to chemical nematicides used in the control of RKNs as more economical and environmentally friendly applications. It is essential to conduct comprehensive studies including pot and field trials in the future regarding the research subject.

## Acknowledgment

This study was conducted at Düzce University Faculty of Agriculture Department of Plant Protection and Department of Agricultural Biotechnology laboratories and there is no conflict of interest among the authors.

## References

- Ahmed, S. & M. Grainge, 1988. Handbook of Plants with Pest Control Properties, John Wiley & Sons Limited, New York, 470 pp.
- Caboni, P., M. Saba, G. Tocco, L. Casu, A. Murgia, A. Maxia, U. Menkissoglu-Spiroudi & N. Natalli, 2013. Nematicidal activity of mint aqueous extracts against the Root-knot nematode *Meloidogyne incognita*. Journal of Agricultural and Food Chemistry, 61 (41): 9784-9788.
- Daferera, D. J., B. N., Ziogas & M. G. Polissiou, 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protection, 22 (1): 39-34.

- Fahey, J. W., A. T. Zalcmann & P. Talalay, 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 56 (1): 5-51.
- Feldmesser, J. & W. A. Feder, 1955. Techniques included in nematocide screening. *Proceedings of the Florida State Horticultural Society*, 68 (1): 108-107.
- Gürsoy, M., A. Balkan & H. Ulukan, 2013. Bitkisel üretimde allelopati. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, 27 (2): 115-122 (in Turkish with abstract in English).
- Moens, M., R. N. Perry & J. L. Starr, 2009. "Meloidogyne Species-A Diverse Group of Novel and Important Plant Parasites, 1-17". In: *Root-Knot Nematodes* (Eds. R. N. Perry, M. Moens & J. L. Starr). CABI Wallingford UK, 321 pp.
- Oka, Y., S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv & Y. Spiegel, 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology*, 90 (7): 710-715.
- Öncüer, C., 2000. *Tarımsal Zararlılarla Savaş Yöntem ve İlaçları* (4. Baskı). Adnan Menderes Üniversitesi Yayınları, No:13: 333 pp (in Turkish).
- Prakash, A. & J. Rao, 1996. *Botanical Pesticides in Agriculture*. CRC Pres, Lewis Publishers, 461 pp.
- SAS Institute, 2013. *JMP 11 Design of Experiments Guide*. SAS Institute.
- Taylor, A. L. & J. N. Sasser, 1978. *Biology, Identification and Control of Root-knot nematodes (Meloidogyne spp.)*. Cooperative Publication of the Department of Plant Pathology, North Caroline State University, and U. S. Agency for International Development, North Carolina State University Graphics, Raleigh, NC, USA, 111 pp.
- Thorne, G., 1961. *Principles of Nematology*. Mc Graw-Hill Book Company Inc., New York, USA, 553 pp.
- Walker, J. T. & J. B. Melin, 1996. *Mentha x piperita*, *Mentha spicata* and effects of their essential oils on *Meloidogyne* in essential oils. *Journal of Nematology*, 28 (4): 629-635.
- Whitehead, A. G., 1997. *Plant Nematode Control*. CAB International, Wallingford, UK, 384 pp.
- 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.