Türk. entomol. derg., 2022, 46 (2): 131-138
DOI: http: //dx.doi.org/10.16970/entoted.1061222

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

The effect of three plant extracts on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)

Üç bitki ekstraktının *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerindeki etkinliği

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Abstract

The use of plant extracts for biotechnical control root-knot nematodes is increasingly important. In this study, the efficacy of three plant extracts, *Thymbra spicata* L. (Lamiales: Lamiaceae) (Mediterranean thyme), *Plantago lanceolata* L. (Lamiales: Plantaginaceae) (ribwort plantain) and *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) (Rosemary), for controlling of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) was examined. The experiments were conducted twice with four replicates under controlled conditions at the Laboratories of Plant Protection Central Research Institute of the Ministry of Agriculture of Turkish Republic in Ankara, Türkiye in 2020. The extracts were prepared at 1.2, 2.5 and 5%. The egg mass number, plant height, fresh and dry plant weight, and fresh and dry root weight of each plant were evaluated in the pot trials. The lowest number of in egg masses was with *T. spicata* (100 ± 2.3) followed by *R. officinalis* then *P. lanceolata* with (104 ± 2.6) and (113 ± 2.1). The least effect was observed at 1.2% concentration of *P. lanceolata* (217 ± 5.5).

Keywords: *Meloidogyne incognita,* plant extract, *Plantago lanceolata*, *Rosmarinus officinalis, Thymbra spicata*

Öz

Kök-ur nematodlarının biyoteknik mücadelesinde bitki ekstraktlarının kullanımı giderek daha fazla önem kazanmaktadır. Bu çalışmada 3 bitki ekstraktı *Thymbra spicata* L. (Lamiales: Lamiaceae) (Zahter, Kekik), *Plantago lanceolata* L. (Lamiales: Plantaginaceae) (Dar yapraklı sınırlı ot) ve *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) (biberiye)'nin *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)'nın kontrolündeki etkinliği araştırılmıştır. Denemeler 2020 yılında Zirai Mücadele Merkez Araştırma Enstitüsü Müdürlüğü Laboratuvarlarında (Türkiye Cumhuriyeti Tarım ve Orman Bakanlığı, Ankara, Türkiye) kontrollü koşullarda 4 tekerrürlü ve 2 tekrarlı olarak yürütülmüştür. Ekstraktlar (%1.2, 2.5 ve 5 dozlarda hazırlanmıştır. Sera-saksı denemelerinde yumurta paketi sayısı, bitki boyu, bitki taze ve kuru ağırlığı, köklerin taze ve kuru ağırlığı değerlendirilmiştir. En düşük yumurta paketi oluşumu *T. spicata 'da* meydana gelirken (100 ± 2.3) bunu *R. officinalis* (104 ± 2.6) ve 113 ± 2.1 ile *P. lanceolata* izlemiştir. En düşük etki ise *P. lanceolata*'nın %1.2'lik konsantrasyonunda (217 ± 5.5) görülmüştür.

Anahtar sözcükler: *Meloidogyne incognita*, bitkisel ekstrakt, *Plantago lanceolata*, *Rosmarinus officinalis, Thymbra spicata*

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Received (Alınış): 21.01.2022 Accepted (Kabul ediliş): 01.05.2022 Published Online (Çevrimiçi Yayın Tarihi): 14.05.2022

Introduction

Plant parasitic nematodes are of great importance among the various agricultural production problems. The root-knot nematodes (*Meloidogyne* spp.) are the most important of all plant parasitic nematodes due to the damage they cause to their hosts (Javed et al., 2006). The root-knot nematodes are found commonly in the greenhouse vegetable cultivation areas especially on the coastal regions of Türkiye. This group has a large host range and causes large yield loses in vegetables all over the world (e.g., 42-54% in tomato and 30-60% in eggplant) (Netscher & Sikora, 1990). Highly toxic nematicides can be used for the control of the root-knot nematodes. The difficulties of controlling with these pests are increasing and some nematicides have been banned. Although nematicides are the main method of control; the increasing concerns for human health and the environment have led to the banning of nematicides and fumigants like methyl bromide, 1,3-dicloropropen, carbofuran and aldicarb. Therefore, alternative control methods need to be developed and applied. Studies on the use of volatile oils, plant extracts and plant-based compounds obtained from plants for controlling pests, have gained momentum and useful results have been obtained (Lee et al., 2001a, b; Varma & Dubey, 2001; Lee & Annis, 2004; Polatoğlu et al., 2015a, b). Plants have several defense mechanisms for pests. Among these mechanisms, various secondary metabolites synthesized within the living organisms have an important place. Secondary metabolites are important chemical compounds that are not primarily related to the vital activities of the plant, and some can provide defense against herbivores (Taiz & Zeigeri, 2002). The compounds with insecticidal and behavioral effects against pests can be classified as alkaloids, glycosides, phenols, terpenoids, tannins and saponins (Shanker & Solanki, 2000; Güncan & Durmuşoğlu, 2004). The use of herbal extracts against root-knot nematodes is widely practiced and there are many studies on the subject (Malik et al., 1987; Taba et al., 2008) In these studies, many plants in 57 families have been found to have nematicidal effects and these families include Lamiaceae, Asteraceae, Rutaceae and Lauraceae (Sukul, 1992; Andres et al., 2012; Kepenekci & Sağlam, 2015).

In this study, the effects of three plant extracts obtained from three plant species that grow naturally in Türkiye on *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) were determined under controlled conditions. The plants were *Thymbra spicata* L. (Lamiales: Lamiaceae) (Mediterranean thyme), *Plantago lanceolata* L. (Lamiales: Plantaginaceae) (ribwort plantain) and *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) (rosemary).

Materials and Methods

Plant material

Thymbra spicata, *P. lanceolata* (from Erzurum) and *R. officinalis* (from Muğla) were collected in Anatolia, Türkiye, during flowering in June and September in the year. Aerial parts of the plants dried in the shade and ground in a grinder.

Extraction of plant material extract

Plant leaves were collected and spread on polythene sheets in the laboratory for 10 days for air drying. The plants were then dried at 80°C for 3-4 days. The dried materials were ground into a fine powder in a blender. Ethanol was added to the ground plant material and shaken in a rotary shaker at 120 rpm for 48 h. The ethanol was filtered and vacuumed in a rotary evaporator at 50-60°C to obtain organic crude extracts free of ethanol (Brauer & Devkota, 1990). Each plant extract was used immediately in all tests. Concentrations of 1.2, 2.5 and 5.0% was prepared in distilled water (Orisajo et al., 2007).

Nematode culture

Pure cultures of *M. incognita* were grown on tomato cv. Tueza F1. The infected roots were washed carefully washed under tap water and egg masses collected from the roots under the microscope and left

to hatch at room temperature. The juveniles that hatched in the first 24 h were discarded and subsequent hatching was monitored and juveniles were collected daily. The suspension with nematodes obtained was adjusted to 1,000 juveniles ml^{-1}.

Greenhouse pot trials

Tomato seeds were placed in 3% NaOCI for 1 min for surface sterilization, then washed with sterile water and dried on blotting paper before planting in tubes containing peat. The tubes were placed in a climate room (25 ± 2ºC, 14:10 h L:D photoperiod). Tomato plants with two to four leaves in the climate room were transplanted into pots (760 ml, 10 x 10 x 11 cm) containing sterile soil-sand mixture (2:1) with one seedling per pot. After 15 days, when the seedlings reached ~10 cm, *M. incognita* larvae were inoculated by pipette into 2 cm deep holes around each seedling at 1,250 juveniles ml^{-1}. The plant extracts were applied in different 2-cm deep holes around each seedling. One ml of extract was applied per plant or pot. Water was used in the controls. The experiments included four replicates and were conducted twice under controlled room conditions. A single control group was used because all trials were set up on the same day and under the same conditions. Eight weeks after the applications, the plants were harvested by cutting 1 cm above the soil surface. The number of egg masses per plant, shoot height, shoot fresh and dry weight, root fresh and dry weight were determined. Before counting the egg mass, the roots were soaked in a aqueous solution of red food-coloring (0.15 g L^{-1}) for 15-20 min, washed and counted under the microscope (Fenner, 1962; Dickson & Struble, 1965; Holbrook et al., 1983). For dry weight determination the samples dried in an oven at 70ºC for 48 h (Mohammad et al., 2007).

Statistical analysis

Statistical analyzes were made using the MINITAB 18 package program. Analyzed by Tukey's multiple comparison test.

Results and Discussion

The effects of three concentrations of *T. spicata*, *P. lanceolata* and *R. officinalis* on the number of egg mass, shoot and root fresh and dry weights are given in Table 1.

There was a decrease in egg mass formation with all three plant extracts compared to the control (Table 1) The different extract concentrations caused a different rate of decrease in egg mass formation compared to the control, and this was statistically significant ($P < 0.05$) (Table 1). Among the doses used, the greatest effect was seen at the highest dose of 5% in all three extracts. *Thymbra spicata* extract that caused the greatest decrease in egg mass formation, followed by *R. officinalis* and *P. lanceolata*, respectively. The lowest effect was observed at 1.2% concentration of *P. lanceolata*

There was an increase in shoot fresh weight for all extracts, but no dose effect, compare to the control (Table 1). However, this effect was not evident for shoot dry weight. The effect on root fresh weight was less consistent. Only *T. spicata* differed from the control. Across extract concentrations, there were some slight suppression in root fresh weight with the extracts at 2.5%. These differences were not evident when root dry was determined. There was not statistically significant effect on shoot height.

Table 1. Effective of three concentrations of *Thymbra spicata, Plantago lanceolata* and *Rosmarinus officinalis* extract on *Meloidogyne incognita* egg mass production and plant growth at three concentrations

Parameters	Extract	1.2%	2.5%	5%
Egg masses	*T. spicata*	195 ± 6.2 b[1]A[2]	128 ± 6.1 bc	100 ± 2.3 bC
	P. lanceolata	217 ± 5.5 bA	149 ± 4.8 bB	113 ± 2.1 bC
	R. officinalis	199 ± 5.5 bA	121 ± 3.7 cB	104 ± 2.6 bC
	Control	309 ± 18.6 aA	309 ± 18.6 aA	309 ± 18.6 aA
		F[3] = 32.4; P < 0.05	F = 104; P < 0.05	F = 207; P < 0.05
Shoot fresh weight (g)	*T. spicata*	8.0 ± 0.4 aA	7.9 ± 0.4 aA	8.1 ± 0.5 aA
	P. lanceolata	6.9 ± 0.2 aA	6.8 ± 0.3 aA	7.2 ± 0.4 aA
	R. officinalis	7.1 ± 0.4 aA	7.3 ± 0.3 aA	7.6 ± 0.4 aA
	Control	4.8 ± 0.4 bA	4.8 ± 0.4 bA	4.8 ± 0.4 bA
		F = 7.63; P < 0.05	F = 10.4; P < 0.05	F = 6.79; P < 0.05
Shoot dry weight (g)	*T. spicata*	1.9 ± 0.1 aA	1.8 ± 0.1 aA	2.8 ± 0.1 aA
	P. lanceolata	1.7 ± 0.2 aA	1.6 ± 0.2 aA	1.8 ± 0.2 aA
	R. officinalis	1.6 ± 0.1 aA	1.6 ± 0.1 aA	1.1 ± 0.1 aA
	Control	1.5 ± 0.1 aA	1.5 ± 0.1 aA	1.5 ± 0.1 aA
		F = 0.90; P > 0.05	F = 0.42; P > 0.05	F = 2.65; P > 0.05
Root fresh weight (g)	*T. spicata*	9.1 ± 0.6 aA	6.8 ± 0.4 aB	9.8 ± 0.3 aA
	P. lanceolata	7.4 ± 0.7 aAB	5.1 ± 0.5 aB	8.6 ± 0.3 abA
	R. officinalis	7.5 ± 0.3 aB	6.1 ± 0.4 aC	8.9 ± 0.2 abA
	Control	7.5 ± 0.8 aA	7.5 ± 0.8 aA	7.5 ± 0.8 bA
		F = 1.87; P > 0.05	F = 1.57; P > 0.05	F = 6.62; P < 0.05
Root dry weight (g)	*T. spicata*	2.4 ± 0.2 aA	1.5 ± 0.1 aB	2.3 ± 0.1 aA
	P. lanceolata	1.9 ± 0.1 abA	1.3 ± 0.1 aB	2.0 ± 0.1 aA
	R. officinalis	1.9 ± 0.1 abA	1.4 ± 0.1 aB	2.1 ± 0.1 aC
	Control	1.6 ± 0.2 bA	1.6 ± 0.2 aA	1.6 ± 0.2 bA
		F = 3.90; P < 0.05	F = 0.51; P > 0.05	F = 7.26; P < 0.05
Shoot height (cm)	*T. spicata*	34.0 ± 1.7 aA	31.0 ± 3.0 aA	31.9 ± 1.9 aA
	P. lanceolata	31.2 ± 1.5 aA	28.1 ± 1.9 aA	27.5 ± 1.1 aA
	R. officinalis	30.7 ± 1.4 aA	26.3 ± 1.7 aA	29.0 ± 1.3 aA
	Control	28.6 ± 1.1 aA	28.6 ± 1.1 aA	28.6 ± 1.1 aA
		F = 1.79; P > 0.05	F = 0.83; P > 0.05	F = 1.75; P > 0.05

[1] Means followed but the same lowercase letter within columns are not significantly different (P < 0.05, Tukey test).
[2] Means followed but the same uppercase letter within rows are not significantly different (P < 0.05, Tukey test).
[3] F value degrees of freedom were all 3,31.

In the study of Oka et al. (2000), in which the nematicidal activity of volatile oils obtained from 27 spices was investigated in laboratory and controlled room conditions, *Carum carvi* L., *Foeniculum vulgare* Mill. (Apiales: Apiaceae), *Mentha longifolia* (L.) Huds. and *M. spicata* L. (Lamiales: Lamiaceae) had the greatest effect under the laboratory conditions at a dose of 1,000 µl L^{-1}. A mixture of *Origanum vulgare* L., *Thymus syriacus* Boiss., and Thymbra capitata (L.) Cav. (Lamiales: Lamiaceae) reduced gall numbers in the roots of cucumber at doses of 100 and 200 mg/kg.

Aydınlı & Mennan (2014) investigated the effectiveness of 12 plant extracts, including *Viscum album* L. (Santalales: Viscaceae), *R. officinalis, Cirsium arvense* (L.) Scop. (Asterales: Asteraceae), prepared in cold and hot form, on *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) under laboratory and controlled room conditions. Cold extracts, except *R. officinalis*, caused higher larval mortality and lower egg hatching than hot extracts, and that the cold extract of *C. arvense* completely inhibited egg hatching. A study was conducted to evaluate the activity of the extract of *R. officinalis* in forming resistance to *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 *(*Tylenchida: Meloidogynidae) and *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev *&* Schuurmans Stekhoven, 1941 (Tylenchida: Pratylenchidae) in soybean. Herbal volatile oil prepared at 0, 1, 2 and 3% were applied to the upper parts of the plants once every 2 weeks and once per month. Rosemary oil was phytotoxic to soybean and caused significant decrease in plant height, shoot fresh and dry weight, and root fresh weight of plants that treated every 2 weeks (Mattei et al., 2013). In another study with *R. officinalis*, activity on *M. incognita* was investigated in soybean cvs CD206 and CD215 under controlled conditions. Rosemary extract prepared at three concentrations (1, 5 and 10%) was applied to the plants weekly. The number of eggs in the root and soil, the number of infective juveniles, the number of knots on the roots and the reproduction factor were evaluated. Rosemary extract reduced egg hatching. Likewise, while the number of galls on cv. CD206 decreased by 46.5%, there was a decrease in both galling and the number of juveniles in the soil with cv CD215 (Müller et al., 2016). Bajestani et al. (2017) tested the activity of marigold (*Tagetes* spp.) and rosemary (*R. officinalis*) and black cumin (*Nigella sativa* L. (Ranunculales: Ranunculaceae) on *M. javanica* on tomato plant. There was a significant decrease in root infection rate and root weight in all applications. Rosemary extract gave the greatest effect with a 40% reduction in the nematode population compared to the control.

Kepenekci & Sağlam (2015) investigated the nematicidal effects of the extracts of five plants [pepper, *C. frutescens*); henbane, *Hyoscyamus niger* L. (Solanales: Solanaceae) ; bead tree, *Melia azedarach* L. (Sapindales: Meliaceae); common cocklebur, *Xanthium strumarium* L. (Asterales: Asteraceae); and yarrow *Achillea santolinoides* subsp. *wilhelmsii* (K.Koch) Greuter (Asterales: Asteraceae)]) on *M. javanica*. Extract concentrations of 3.6% and 12% for *H. niger*, *X. strumarium* and *M. azedarach* completely inhibited hatching, but the effect was less with by *C. frutescens* and *A. santolinoides* subsp. *wilhelmsii*. Considering the mortality rate of infective juveniles, the most effective extracts were from *X. strumarium* and *M. azedarach*.

In another study, the efficacy of eucalyptus [*Eucalyptus camaldulensis* Dehnh. (Myrtales: Myrtaceae)], garlic [*Allium sativum* L. (Asparagales: Amaryllidaceae)], marigold [*Tagetes erecta* L. (Asterales: Asteraceae)] and neem tree [(*Azadirachta indica* A. Juss. (Sapindales: Meliaceae)] extracts and volatile oils on *M. incognita* was tested under laboratory, field and greenhouse conditions. Neem extract provided the highest mortality rate (65%) in laboratory conditions. This was followed by neem volatile oil (64%) and marigold extract (61%), respectively. Likewise, the most effective results were obtained in the applications of neem extract 44% and essential oil 33% under greenhouse and field conditions (Kamal et al., 2009).

In another study, the effects of aqueous extracts of *Euphorbia myrsinites* L. (Malpighiales: Euphorbiaceae) and *Drimia maritima* (L.) Stearn (Asparagales: Asparagaceae) on the damage of *M.*

incognita were evaluated. All dilutions of the extracts (1, 2 and 4%) applied to the soil, reduced root galling caused by *M. incognita* and increased tomato yield compared to the control (Kaşkavalcı & Civelek, 2009). Leaves of the castor oil plant [*Ricinus communis* L. (Malpighiales: Euphorbiaceae)] and oleander [*Nerium oleander* L. (Gentianales: Apocynaceae)], fruit of squirting cucumber [*Ecbalium elaterium* (L.) A.Rich. (Cucurbitales: Cucurbitaceae)] and whole plants of marigold (*T. erecta*) was tested on root-knot nematode (*M. incognita*). All treatments resulted in a less galling compared to a positive control (Hatipoğlu & Kaşkavalcı, 2007).

The nematicidal effect of methanol extracts of *Sylibum marianum* (L.) Gaertn. (Asterales: Asteraceae), *P. lanceolata* and *Cassia fistula* L. (Fabales: Fabaceae) on *M. incognita* was tested under laboratory conditions. *Plantoago lanceolata* was the most effective extract and reduced egg hatching by 75% and killed infective juveniles within 5 days (Adekunle et al., 2006). In another study conducted by Kepenekci et al. (2017), three concentrations (3, 6 and 12%) of the extracts obtained from *Capsicum frutescens* L. (Solanales: Solanaceae), *H. niger*, *X. strumarium*, *A. antolinoides subsp. wilhelmsii* and *M. azedarach* were tested against root-knot nematodes (*M. incognita* race 2 and *M. arenaria* race 2). As a result of the study, 12% concentrations of *H. niger* and 12% of *X. strumarium* concentrations were found to be effective on egg hatching of *M. arenaria* the concentration of 12% of *X. strumarium* was effective on egg hatching. A concentration of 12% of *M. azedarach* was effective against juveniles of *M. arenaria* and *M. incognita*.

Considering all studies, it is clear that some plant extracts give effective results against root-knot nematodes. In the present study, it was found that *T. spicata*, *P. lanceolata* and *R. officinalis* can be effective against root-knot nematodes under controlled conditions. It is therefore possible that these plant extracts could be of valued in the control programs against root-knot nematodes, but further studies are needed to confirm their value under field conditions.

Acknowledgments

Prof. Dr. Şaban Kordalı (Muğla Sıtkı Koçman University, Muğla, Türkiye) is thanked for provision of the plant extracts, Prof. Dr. İlker Kepenekci (Gaziosmanpaşa University, Tokat, Türkiye) for his help during the establishment of the experiments and Assoc. Prof. Dr. Mustafa Alkan (Directorate of Plant Protection Central Research Institute, Ankara, Türkiye) for help with statistical analysis.

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