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Molecular Identification of Root-knot Nematode Species (*Meloidogyne* spp.) on Lavender of Isparta and Burdur Provinces in Turkey

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ABSTRACT

This study was conducted to identify and to determine the distribution of root-knot nematode species in total of 625 ha of Laventer (*Lavandula × intermedia* Emeric ex Loisel. var. Super) cultivated area of Isparta and Burdur Provinces of Turkey. A total of 60 samples were collected in autumn of 2020. Root knot nematode species molecular identification was determined by species-specific primers from egg masses. The 17 samples taken from cultivated lavender fields were found to be infected with Root knot nematode. As a result of molecular identification, 12 of samples were found to be *Meloidogyne incognita*, while 5 of them were found to be *M. arenaria*. This was the first report of infestation of lavender by *M. incognita* in Turkey. Of studied areas, Keçiborlu district of Isparta Province with most cultivated lavender area sustained 7 samples of *M. incognita* and 3 of samples *M. arenaria*.

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Türkiye'nin Isparta ve Burdur İllerinin Lavantalarında Kök-ur Nematod Türlerinin (*Meloidogyne* spp.) Moleküler Tanımlanması

ÖZET

Bu çalışma, Türkiye'nin Isparta ve Burdur illerinde Lavanta (*Lavandula × intermedia* Emeric ex Loisel. var. Super) yetiştirilen yaklaşık 625 ha'lık bir alanda Kök-ur nematodu türlerinin belirlenmesi ve yayılışlarının saptanması amacıyla yürütülmüştür. 2020 yılının sonbaharında toplam 60 örnek toplanmıştır. Kök-ur nematod türlerinin moleküler tanımlaması, yumurta paketinden türe özgü primerler ile belirlenmiştir. Kültürü yapılan lavanta tarlalarından alınan 17 örneğin Kök ur nematodları ile enfekte olduğu bulunmuştur. Moleküler tanılama sonucunda bu örneklerin 12 tanesinde *Meloidogyne incognita* bulunurken, 5 tanesinde *M. arenaria* saptanmıştır. Bu, Türkiye'de lavantada *M. incognita* enfeksiyonuna ilişkin ilk rapordur. Lavantanın en çok yetiştirildiği Isparta İli Keçiborlu İlçesi'nin Kök-ur nematodu ile enfekte olduğu belirlenmiştir. Keçiborlu İlçesi'nde 7 örnek *M. incognita* tespit edilirken, 3 örnek *M. arenaria* tespit edilmiştir.

Bitki Koruma

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Anahtar Kelimeler Lavanta
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INTRODUCTION

Lavender (*Lavandula* spp.) is an aromatic herb of Lamiaceae. The essential oil obtained from the spikes of the lavender is in great demand around the world which is mostly used in cosmetics, perfumery, flavoring and pharmaceutical industries (Tarhan et al., 2019). Lavender (*L. angustifolia* = *L. officinalis* = *L. vera*), Lavandin (*L. angustifolia* × *L. latifolia* = *Lavandula × intermedia* = *L. hybrida*), and Spike lavender (*L. spica* = *L. latifolia*) are important lavender species (Erbaş et al., 2017). Lavender is densely cultivated in France, Bulgaria, Spain, Italy, Greece, England, Russia, the USA, Austria, and the North African countries worldwide (Tucker, 1985; Erbaş et al., 2017). Lavender is cultivated approximately 1200 ha area in Turkey and in Burdur and Isparta Provinces, 167.8 and 456.8 ha of cultivated area are

reported respectively (TÜİK, 2021). The most lavender cultivation is in Kuyucak village in Keçiborlu district of Isparta Province which consisted of 93% lavender production of Turkey (Başaran, 2017). The largest lavender field in Turkey is in Akçaköy in Yeşilova district in Burdur Province where 37 ha of cultivation are done in a single plot (Anonim, 2021). The cultivated lavender cultivar in Isparta and Burdur provinces is *Lavandula × intermedia* var. Super which has very well adapted particularly to the non-irrigated, arid, and sloping lands of this locality (Erbaş et al., 2017).

Lavender is infected by many pests and pathogens and that might cause significant damage and effect negatively in terms of their quality and essence yields (Gorustovich et al., 1997). Root-knot nematodes (*Meloidogyne* spp.) are the most economically important nematodes in agriculture due to damage to vascular tissues, wide host range, forming disease complexes with soil pathogens (Udo et al., 2008; Moens et al., 2009; Lobna et al., 2016; Siddiqui and Zaki, 2017). *Meloidogyne incognita* (Kofoid and White, 1912) Chitwood, 1949*, M. javanica* (Treub, 1885) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949 and *M. hapla* Chitwood, 1949 are the most dominant species in agricultural and horticultural crops (Hussey and Janssen, 2002; Brito et al., 2008; Sarkar, 2020) and are common in the Mediterranean area (Ornat and Sorribas, 2008; Devran and Söğüt, 2009; Uysal et al., 2017; Gonçalves et al., 2020). *Meloidoyne incognita* was reported in lavender in Argentina (Gorustovich et al., 1997) and Egypt (Ibrahim and Mokbel, 2009) in cultivars *L. hybrida* and *L. officinalis*, respectively. Carneiro et al. (2014) found *M. luci* on *L. spica. Meloidogyne hapla* from Greece (Gonçalves et al., 2020) and *M. arenaria* (Özalp et al., 2020) from Turkey are reported in *L. angustifolia.*

The aim of this study was to identify root knot nematode species in lavender cultivation areas of Isparta and Burdur provinces of Turkey by molecular methods and consequently determine their distribution.

MATERIAL and METHOD

Root-knot nematode sampling locations

Lavender fields in Isparta and Burdur provinces of Turkey were surveyed in autumn of 2020. A total of 60 samples were collected in the study (Table 1). In each field, root and soil samples were taken from lavender localities indicating symptoms of stunting plants. Root samples were placed in a separate bag and brought to the laboratory in a cold chain and stored at 4ºC for further use.

Nematode extraction

Each lavender root samples were gently washed with tap water and examined under a stereomicroscope for

Table 1. Locatians of Root-knot nematode samples
Çizelge 1. Kök-ur nematodu örneklerinin lokasyon bilgileri

Sample no *Örnek no*	Code Kod	Village or smalltown/ District/ Province (*Köy yada Kasaba/ İlçe/ İl)*
1	E1	Sorkuncak/Eğirdir/Isparta
2	E2	Sorkuncak/Eğirdir/Isparta
3	E3	Sorkuncak Eğirdir/Isparta
4	E4	Sarıidris/ Eğirdir/Isparta
5	E5	Sarıidris/ Eğirdir/Isparta
6	E6	Sarıidris/ Eğirdir/Isparta
7	E7	Eğirdir/Isparta
8	ISP1	Centre/Isparta
9	ISP2	Centre/Isparta
10	KL1	Kılıç/Keçiborlu/Isparta
11	KL2	Kılıç/Keçiborlu/Isparta
12	KL3	Kılıç/Keçiborlu/Isparta
13	KL4	Kılıç/Keçiborlu/Isparta
14	A1	Aydoğmuş/Keçiborlu/Isparta
15	A2	Aydoğmuş/Keçiborlu/Isparta
16	A3	Ardıçlı/Keçiborlu/Isparta
17	A4	Ardıçlı/Keçiborlu/Isparta
18	C1	Çukurören/Keçiborlu/Isparta
19	C2	Çukurören/Keçiborlu/Isparta
20	C3	Çukurören/Keçiborlu/Isparta
21	C4	Çukurören/Keçiborlu/Isparta
22	C5	Çukurören/Keçiborlu/Isparta
23	C6	Çukurören/Keçiborlu/Isparta
24	C7	Çukurören/Keçiborlu/Isparta
25	C8	Çukurören/Keçiborlu/Isparta
26	C9	Çukurören/Keçiborlu/Isparta
27	K1	Kuyucak//Keçiborlu/Isparta
28	K2	Kuyucak//Keçiborlu/Isparta
29	K3	Kuyucak//Keçiborlu/Isparta
30	K4	Kuyucak//Keçiborlu/Isparta
31	K5	Kuyucak//Keçiborlu/Isparta
32	K6	Kuyucak//Keçiborlu/Isparta
33	K7	Kuyucak//Keçiborlu/Isparta
34	K8	Kuyucak//Keçiborlu/Isparta
35	K9	Kuyucak//Keçiborlu/Isparta
36	K10	Kuyucak//Keçiborlu/Isparta
37	K11	Kuyucak//Keçiborlu/Isparta
38	K12	Kuyucak//Keçiborlu/Isparta
39	K13	Kuyucak//Keçiborlu/Isparta
40	K14	Kuyucak//Keçiborlu/Isparta
41	K15	Kuyucak//Keçiborlu/Isparta
42	K16	Kuyucak//Keçiborlu/Isparta
43	K17	Kuyucak//Keçiborlu/Isparta
44	K18	Kuyucak//Keçiborlu/Isparta
45	K19	Kuyucak//Keçiborlu/Isparta
46	K20	Kuyucak//Keçiborlu/Isparta
47	S1	Boğazköy/Sütçüler/Isparta
48	S2	Boğazköy/Sütçüler/Isparta
49	B1	Centre/Burdur
50	B2	Centre/Burdur
51	B3	Akçaköy/Yeşilova/Burdur
52	B4	Akçaköy/Yeşilova/Burdur
53	B5	Akçaköy/Yeşilova/Burdur
54	B6	Akçaköy/Yeşilova/Burdur
55	B7	Akçaköy/Yeşilova/Burdur
56	B8	İlyas/Yeşilova/Burdur
57	B9	İlyas/Yeşilova/Burdur
58	B10	İlyas/Yeşilova/Burdur
59	B11	Salda/ Yeşilova/Burdur
60	B12	Salda/ Yeşilova/Burdur

evidence of galls. Then, egg masses and mature females were collected from infested roots using needle and placed in Eppendorf tubes under a stereomicroscope.

Molecular identification

DNA extraction from nematode isolates was performed following cetyl trimethyl ammonium bromide (CTAB) method with slight modifications (El-Qurashi et al., 2017; Mondino et al., 2015). Two species-specific primers were used in the PCR amplifications, which was conducted by thermocycler (Veriti Thermal cycler, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) in a total volume of 25 µL (Table 2). Reaction mixture consisted of 10 ng DNA (5 µl), PCR buffer (2.5 µl), 2 mM MgCI2 (1 µl), 0.2 mM dNTP (1 µl), 10 mM Primer F (1 µl), 10 mM Primer R (1 µl), 1 unit Taq DNA polymerase (GenEon, San Antonio, TX, USA) (0.25 µl) and ddH2O (13.25 µl). PCR cycles; initial denaturation at 94°C for 3 min, followed by 35 cycles each consisting of 30 sec at 94°C, 30 sec at 56°C and 60 sec at 72°C for Far/Rar and 94°C for 3 min for INCK14F/INCK14R primers, followed by 30 sec at 94°C, 30 sec at 60°C and 60 sec at 72°C with a final extension at 72°C for 7min.

PCR products were separated using agarose electrophoresis in 2% gel (Agarose Type I, Sigma-Aldrich, St. Louis, MO, USA) with ethidium bromide. The gel was run for 2 hours using constant voltage of around 90 V and then visualized and photographed under UV light using a gel documentation system. The specific band was detected for each SCAR marker separately.

Table 2. Species specific primers of root-knot nematodes for molecular identification
Çizelge 2. Moleküler tanımlama için kök-ur nematodlarının türe özgü primerleri

Nematode species *Nematod türü*	*Primers* *Primer*	*Primer sequences (5-3)* *Primer sekansları*	*Fragments (bp)* *Uzunlukları*	*Reference* *Kaynak*
M. arenaria	FAR	TCGGCGATAGAGGTAAATGAC	420	Zijlstra vd., 2000
	RAR	TCGGCGATAGACACTACAACT		
M. incognita	INCK14R	CCCGCTACACCCTCAACTTC	399	Randing vd., 2002
	INCK14F	GGGATGTGTAAATGCTCCTG		

RESULT and DISCUSSION

Root knot nematode infested plant roots were found in 17 (28.3%) of 60 samples collected from lavender fields in Isparta and Burdur provinces. Root knot nematodes were found in 3 of 12 samples in Burdur Province and 14 of 48 samples in Isparta Province (Table 3).

As a result of the molecular analysis, while 12 of the 17 samples with Root knot nematode were identified as *Meloidogyne incognita* (Figure 1), 5 of them were identified as *M. arenaria* (Figure 2). *Meloidogyne arenaria* were detected in one sample (B12) in Burdur Province and four samples (E1, A3, K5, K17) from Isparta Province. Only two samples of *M. incognita* (B3 and B7) were found in Yeşilova district in Burdur Province whereas in Isparta Province, 10 samples of *M. incognita* (C5, C9, K4, K11, K16, K20, E4, ISP1, KL3, S1) were identified. Seven samples of *M. incognita* and 3 samples of *M. arenaria* were determined in intensive lavender cultivated Keçiborlu district (Table 3).

Table 3. Number of infested samples with Root knot nematode in lavender fields
Çizelge 3. Lavanta tarlalarında Kök ur nematodu ile enfekte olmuş örnek sayısı

Province *İl*	*District* *İlçe*	*Number of samples* *Örnek sayısı*	*Number of samples with nematodes* *Nematod ile bulaşık örnek sayısı*	*Meloidogyne incognita*	*Meloidogyne arenaria*
Isparta	Eğirdir	7	2	1	1
	Centre	2	1	1	x
	Keçiborlu	37	10	7	3
	Sütçüler	2	1	1	x
Burdur	Centre	2	x	x	x
	Yeşilova	10	3	2	1
Total		60	17	12	5

In Turkey, Lavender is most cultivated in Keçiborlu district of Isparta province and no study has been found on the detection of root knot nematode in this region. In the study, it was determined that Keçiborlu district was significant locality infected with root-knot nematode. It was confirmed that tomatoes were grown before lavender in the C5 and K20 sampling areas of Keçiborlu district where *M. incognita* was detected. On the other hand, it was found that potatoes were grown before lavender in B3 and B7 sampling areas in

Yeşilova district where *M. incognita* was detected. Previously, *M. arenaria* was detected in lavender fields of Edirne and Kırklareli in Turkey (Özalp et al., 2020). However, there is no report of *M. incognita* infecting lavender in Turkey. The present study is the first report of *M. incognita* in lavender in Turkey by using molecular markers. Moreno et al. (1990) reported that lavender species were a suitable host for *M. arenaria*. *Meloidoyne incogita* was found infecting *L. hybrida* Rev. from Argentina (Gorustovich et al., 1997) and *L. officinalis* Chaix et Vill. from Egypt (Ibrahim and Mokbel, 2009).

Figure1. PCR products amplified using primers INCK14R/INCK14F
Şekil 1. INCK14R/INCK14F primerleri kullanılarak çoğaltılmış PCR ürünleri

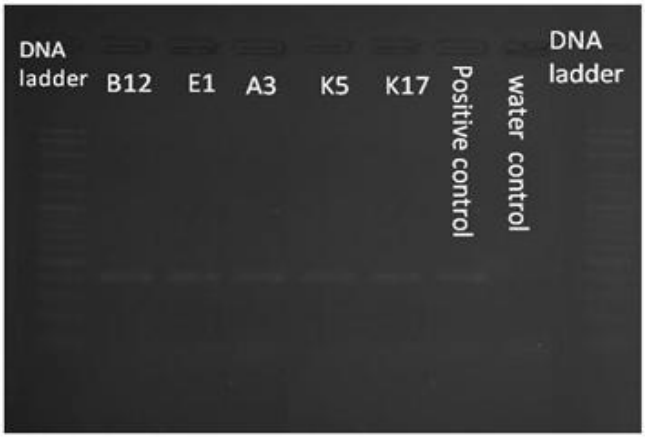

Figure2. PCR products amplified using primers FAR/RAR
Şekil 2. FAR/RAR primerleri kullanılarak çoğaltılmış PCR ürünleri

CONCLUSION

In conclusion, this study showed the detection of root knot nematode infestation in the lavender fields of Isparta and Burdur provinces. *M. incognita* was more common nematode species in studied area. It is necessary to pay attention to lavender seedlings transportion to prevent the dispersal of nematodes from this region to other regions of the country. In addition, weeds that are known to be host to root knot nematodes should be controlled in the fields. Newly to be established lavender field should have soil analyses before starting production with a nematode resistant variety.

Conflict of Interest

The author declare that does not have any competition and any conflicts of interest.

REFERENCE

Anonymus 2021. Türkiye'nin En Büyük Lavanta Bahçesi, Akçaköy Lavanta. Http://Www.Burdur.Gov.Tr. Erişim Tarihi:22.06.2021.

Başaran, N. 2017. Bringing Lavander to Economy in Rural Development and Rural Tourism Scope. International Journal of Agricultural and Natural Sciences, 10(1):47-49.

Brito JA, Kaur R, Cetintas R, Stanley JD, Mendes ML, Mcavoy EJ, Dickson DW 2008. Identification and Isozyme Characterisation of Meloidogyne spp. Infecting Horticultural and Agronomic Crops, and Weed Plants in Florida. Nematology, 10(5): 757-766.

Carneiro Rmdg, Correa Vr, Almeida Mra, Gomes Acmm, Mohammad Deimi A, Castagnone – Sereno P, Karssen G 2014. *Meloidogyne Luci* N.Sp. (Nematoda: Meloidogynidae), A Root-Knot Nematode Parasitizing Different Crops in Brazil, Chile nd Iran. Nematology, 16: 289-301.

Devran Z, Söğüt MA 2009. Distribution and İdentification of Root-Knot Nematodes from Turkey. Journal of Nematology, 41 (2): 128-133.

El-Qurashi MA, El-Zawahry AM, Abd-El-Moneem KMH, Hassan MI 2017. Morphological and Molecular Identification of Root-Knot Nematodes Infecting Pomegranate in Assiut Governorate, Egypt. Journal of Phytopathology and Pest Management, 30-37.

Erbaş S, Kucukyumuk Z, Baydar H, Erdal I, Sanli A 2017. Effects of Different Phosphorus Doses on Nutrient Concentrations as well as Yield and Quality Characteristics of Lavandin (Lavandula× intermedia Emeric Ex Loisel. var. Super). Turkish Journal of Field Crops, 22(1): 32-38.

Gorustovich MNA, Otero MDC, Rossi ER, Boldrini C 1997. Meloidogyne Species in Rosemary (*Rosmarinus officinalis* L.) and Lavender (*Lavandula hybrida* Rev.) in The Cerrillos Department, Province of Salta, Argentina. Iı Wocmap Congress Medicinal and Aromatic Plants, Part 3: Agricultural Production, Post Harvest Techniques, Biotechnology, Pp. 209–12.

Gonçalves Ar, Conceição Il, Kormpi M, Tzortzakakis Ea 2020. *Lavandula angustifolia* and Oxalis Pes-Caprae, Hosts of *Meloidogyne hapla* and *Meloidogyne javanica*-A Note for *Meloidogyne luci* in Greece. Hellenic Plant Protection Journal,13(2):78-82.

Hussey RS, Janssen GJW 2002. Root-Knot Nematodes: Meloidogyne Species. Plant Resistance to Parasitic Nematodes, 43-70.

Ibrahim IK, Mokbel AA 2009. Occurrence and Distribution of The Root-Knot Nematodes Meloidogyne spp. and Their Host Plants in Northern Egypt. Egyptian Journal of Experimental Biology, 5:1–7.

Lobna H, Mayssa C, Hajer R, Ali R, Najet HR 2016. Biocontrol Effectiveness of Indigenous Trichoderma Species Against *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *radicis lycopersici* on Tomato. International Journal of Agricultural and Biosystems Engineering, 10(10):613-617.

Moens M, Perry Rn, Starr Jl 2009. Meloidogyne Species – A Diverse Group of Novel and Important Plant Parasites. In Perry, Rn, Moens M, Starr Lj (Eds). Root-Knot Nematodes. Cabi International, Wallingford, Oxon (Cabı), P. 1-17.

Mondino Ea, Covacevich F, Studdert Ga, Pimentel Jp, Berbara Rl, 2015. Extracting Dna f Nematodes Communities from Argentine Pampas Agricultural Soils. Annals of the Brazilian Academy of Sciences, 87(2): 691–697.

Moreno JE, French EC, Prine GM, Dunn RA 1990. Evaluation of Selected Herbs for Resistance to Root-Knot Nematodes Infection. Proceedings of the 26th Annual Meeting 534–40.

Ornat C, Sorribas FJ 2008. Integrated Management of Root-Knot Nematodes in Mediterranean Horticultural Crops. In Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes (Pp. 295-319). Springer, Dordrecht.

Özalp T, Könül G, Ayyıldız Ö, Tülek A, Devran Z 2020 First report of root-knot nematode, *Meloidogyne arenaria*, on lavender in Turkey. Journal of nematology, 52:1-3.

Randig O, Bongiovanni M, Carneiro RMDG, Castagnone-Sereno, P 2002. Genetic Diversity of Root-Knot Nematodes from Brazil and Development of Scar Marker Specific for the Coffee Damaging Species. Genome, 45: 862-870.

Sarkar S 2020. Incidental Finding of Root Knot Symptoms in *Lavandula angustifolia* Mill: First Report from India. Journal of Medicinal Plants, 8(4): 292-299.

Siddiqui A, Zaki MJ 2017. Efficacy of Some Seeds of Family Apiaceae Against Root Knot Nematode, *Meloidogyne javanica* (Treub) Chitwood. *Int. J. Biol. Biotech*, 14(1):89-94.

Tarhan Y, Açıksöz S, Çelik D 2019. Agriculture of Lavender and Sustainable Development: Isparta/Keçiborlu-Kuyucak Vıllage. Bartın University International Journal of Natural and Applied Sciences, 2 (2): 216-227.

Tucker Ao 1985. Lavender, Spike, and Lavandin. The Herbarist, 51:44-50.

Tüik, 2021. Lavanta Ekim Alanı. https://data.tuik.gov.tr/kategori/getkategori?P=Tarim-111&Dil=1. Erişim Tarihi: 22.06.2021

Udo IA, Uguru MI, Ogbuji RO 2008. Sources of Tolerance to Root-Knot Nematode, *Meloidogyne javanica* in Cultivated and Wild Tomato Species. Plant Pathol. J. 7 (1): 40–44.

Uysal G, Söğüt MA, Elekçioğlu İH 2017. Identification and Distribution of Root-Knot Nematode Species (Meloidogyne spp.) in Vegetable Growing Areas of Lakes Region in Turkey. Turkish Journal of Entomology, 41(1), 105-122.

Zijlstra C, Donkers-Venne DTHM, Fargette M 2000. Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* Using Sequence Characterized Amplified Region (Scar) Based Pcr Assays. Nematology, 8: 847-853.