KSÜ Tarım ve Doğa Derg 25 (Ek Sayı 1): 176-179, 2022
KSU J. Agric Nat 25 (Suppl 1): 176-179, 2022
https://doi.org/10.18016/ksutarimdoga.vi. 1080185

First Report of Root-Knot Nematode, *Meloidogyne arenaria* on Lemon Balm (*Melissa officinalis* L.) in Turkey

Hürkan ATAŞ[1], Gülsüm UYSAL[2], Çiğdem GÖZEL[3], Uğur GÖZEL[4], Zübeyir DEVRAN[5]✉
[1,3,4]Department of Plant Protection, Faculty of Agriculture, University of Çanakkale Onsekiz Mart, Çanakkale, Türkiye, [2]Batı Akdeniz Agricultural Research Institute, Antalya, Türkiye, [5]Department of Plant Protection, Faculty of Agriculture, University of Akdeniz, Antalya, Türkiye
[1]https://orcid.org/0000-0002-5135-527X, [2]https://orcid.org/0000-0003-1722-2518, [3]https://orcid.org/0000-0002-0742-7205
[4]https://orcid.org/0000-0003-1363-1189, [5]https://orcid.org/0000-0001-7150-284X
✉: zdevran@akdeniz.edu.tr

ABSTRACT
Lemon palm (*Melissa officinalis* L.), a perennial plant from Lamiaceae family, is cultivated in all Mediterranean countries and coastal regions of Türkiye. It can be attacked by several pathogens like nematodes which reduce its yield and quality. In this study, morphometric measurements, morphological and molecular identifications were done using juveniles and females obtained from galled roots of lemon balm collected from Balıkesir province of Türkiye. As a result, *M. arenaria* was the only identified species in analyzed samples. This is the first report of *M. arenaria* detected on lemon balm in Türkiye.

Plant Protection

Research Article

Article History
Received : 03.03.2022
Accepted : 21.04.2022

Keywords
Identification
Melissa officinalis
Meloidogyne arenaria

Türkiye'de Melisada (*Melissa officinalis* L.) Bulunan Kök-ur Nematodu *Meloidogyne arenaria*'nın İlk Kaydı

ÖZET
Lamiaceae familyasından çok yıllık bir bitki olan melisa (*Melissa officinalis* L.), tüm Akdeniz ülkelerinde ve Türkiye'nin kıyı bölgelerinde yetiştirilmektedir. Melisa bitkileri nematodlar gibi verim ve kalitede düşüşe yol açan çeşitli patojenler tarafından saldırıya uğramaktadır. Bu çalışma kapsamında Balıkesir ilinden toplanan melisa bitkilerinin urlu köklerinden elde edilen larvalar ve dişi bireyler kullanılarak morfometrik ölçümler, morfolojik ve moleküler tanılamalar yapılmıştır. Sonuç olarak, *M. arenaria*, analiz edilen örneklerde tespit edilen tek tür olmuştur. Bu çalışma Türkiye'de melisa üzerinde *M. arenaria*'nın ilk tespitidir.

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 03.03.2022
Kabul Tarihi : 21.04.2022

Anahtar Kelimeler
Tanılama
Melissa officinalis
Meloidogyne arenaria

Atıf Şekli: Ataş G, Uysal G, Gözel Ç, Gözel U, Devran Z 2022. Türkiye'de Melisada (Melissa officinalis L.) Bulunan Kök-ur Nematodu *Meloidogyne* arenaria'nın İlk Kaydı. KSÜ Tarım ve Doğa Derg 25 (Ek Sayı 1): 176-179. https://doi.org/10.18016/ksutarimdoga.vi. 1080185

To Cite : Ataş G, Uysal G, Gözel Ç, Gözel U, Devran Z 2022. First report of root-knot nematode, *Meloidogyne arenaria* on lemon balm (*Melissa officinalis* L.) in Türkiye. KSU J. Agric Nat 25 (Suppl 1): 176-179. https://doi.org/10.18016/ksutarimdoga.vi.1080185

INTRODUCTION

Medicinal and aromatic plants have been used in pharmaceuticals, perfumery, cosmetics, and food industries and there is a current significant increasing demand in tropical and subtropical regions of the world (Pandey, 2017). *Melissa officinalis* belongs to Lamiaceae family and is distributed in all Mediterranean countries. This plant has many common names such as garden balm, lemon mint, sweet balm in Türkiye (Mill, 1982; Baytop, 1994). This plant is widely cultivated due to its content of aromatic, culinary and medicinal compound (Verma et al. 2015). In addition, it has been known that lemon balm essential oils can be used as antioxidant and antitumoral agents for the treatment of Alzheimer's disease and have a positive effect on human nervous system (Akhondzadeh et al. 2003).

Lemon balm hosts several pests and pathogens which reduce its yield and quality (Bokor et al. 2008). Plant parasitic nematodes, particularly root-knot nematodes, cause serious economic losses in vegetables, horticultural crops, and medicinal and aromatic plants (Koshy et al. 2005; Karssen et al. 2013; Ataş et al. 2021).

Meloidogyne arenaria (Neal, 1889) Chitwood, 1949 was reported on lemon balm in Greece (Karanastasi

et al. 2008). Also, *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949 and *M. arenaria* on *M. officinalis* was reported in Cuba (Kindelan et al. 1990). In several other studies, *Melissa officinalis* was found to be the host of *M. paranaensis* n. sp. (Carneiro et al. 1996), and *M. incognita* race 3 (Walker, 1995; Mendonça et al. 2017). However, no reports were found on root-knot nematodes in lemon balm in Türkiye.

MATERIAL and METHODS

A survey was caried out in lemon balm growing areas in Balıkesir province of Türkiye in 2020. Root samples were taken from plants with wilting and drying symptoms and examined under a binocular microscope. Egg masses were separately taken from roots of lemon balm by a needle. Then, they were incubated and transferred to sieve included water for second stage juveniles (J2s) hatching. Based on Seinhorst's (1959) method, J2s hatched from the egg were fixed in TAF (triethanolamine formalin) for permanent slides. Overall, measurements of 25 J2s were taken place under the Leica DM1000 stereomicroscope as described by Karssen (2002). For morphological diagnosis, females were taken from the roots by a needle and scalpel under binoculars. Females were prepared in glycerin by cutting in 45% lactic acid to obtain perineal patterns (Hooper, 1986). The population was morphologically identified by the comparing to morphometric measurements of Jepson (1987) and Karssen (2002). DNA was extracted from J2s for molecular identification using isolation kit (High Pure PCR Template Preparation Kit, Roche) and were performed with species-specific primers; Inc-K14F/Inc-K14R (Randig et al., 2002), MincF1/MincR1 (Devran et al. 2018), Fjav/Rjav (Zijlstra et al. 2000); Far/Rar (Zijlstra et al. 2000), JMV1/JMV2/JMVhapla (Wishart et al. 2002). PCR reactions were carried out according to our previous studies (Özalp et al. 2020; Ataş et al. 2021).

RESULTS and DISCUSSION

The results of morphometric measurements and morphological identification showed that the overall morphology of this population appeared to be similar to *M. arenaria* (Table 1, Figure. 1).

Table 1. *Morphometric measurements of Meloidogyne arenaria J2s on Melissa officinalis*
Çizelge 1. *Melissa officinalis'den elde edilen Meloidogyne arenaria'ya ait J2s'lerin morfometrik ölçümleri*

Diagnostic characters	*Current study*	*Whitehead, 1968*	*Cliff and Hirschmann, 1985*
Body length	405.31 ± 9.68 (389.67-430.12)	450-490	391.6-605.2
Greatest body width	14.58 ± 1.37 (12.29-18.20)		12.8-17.8
Body width at stylet base	8.69 ± 0.60 (7.12-9.99)		
Body width at anus	9.34 ± 0.82 (7.59-11.34)		9.7-12.8
Stylet length	12.54 ± 1.29 (10.27-13.95)	10	10.1-11.9
DGO	3.34 ± 0.32 (2.68-3.84)	3	2.7-4.7
Tail length	47.61 ± 4.31 (40.87-56.66)		43.6-69.4
Excretory pore to head end	80.08 ± 5.96 (66.25-101.01)		75.0-105.2
Body width at excretory pore	12.15 ± 0.79 (10.79-13.61)		
A	27.28 ± 2.59 (23.15-33.71)	26-32	22.4-40.5
B	3.79 ± 0.23 (3.27-4.22)		
C	8.35 ± 0.72 (7.22-9.93)	6-7.5	7.5-10.9
c'	5.13 ± 0.59 (4.18-6.63)		

Note: All measurements are in µm and in the form: mean ± s.d. (range)

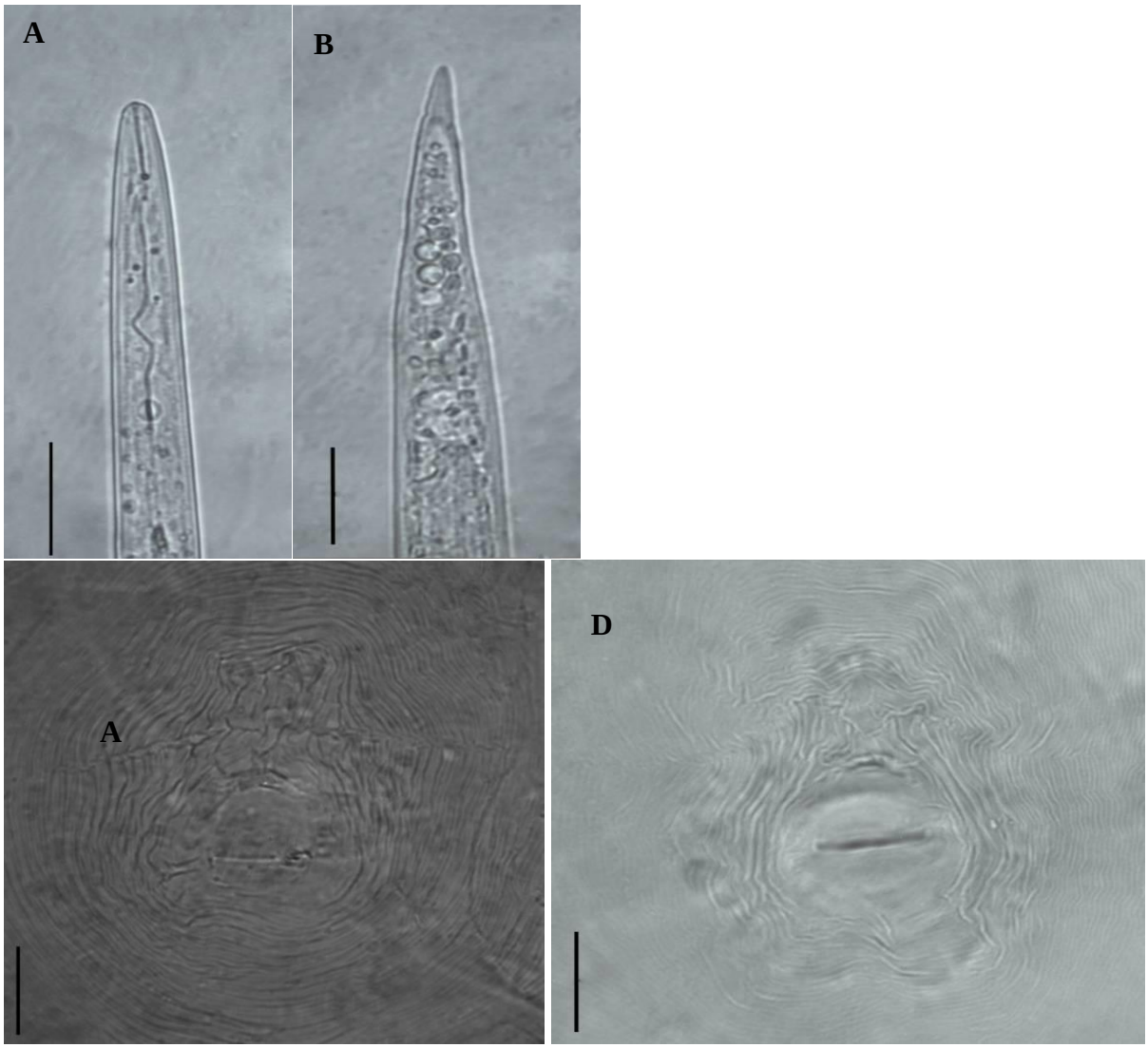

Figure 1. *Meloidogyne arenaria* obtained from *Melissa officinalis* A: Anterior end region, B: Tail region, C-D: Perineal pattern (Scale bar: 20 µm)

Şekil 1. *Melissa officinalis* 'den elde edilen *Meloidogyne arenaria* A: Anterior end region, B: Tail region, C-D: Perineal pattern (Skala bar: 20 µm)

Figure 2. PCR products obtained with *Meloidogyne arenaria*-specific Far/Rar primers. M: DNA Ladder (Hibrigen 100 bp); BM1-BM4: Samples; K18: *M. arenaria* (positive control); W: Water

Şekil 2. *Meloidogyne arenaria*-spesifik Far/Rar primerleri kullanılarak elde edilen PCR ürünleri. M: DNA Ladder (Hibrigen 100 bp); BM1-BM4: Örnekler; K18: *M. arenaria* (pozitif kontrol); W: Su

In molecular identification, PCR with *Meloidogyne arenaria* specific Far/Rar primer sets produced an expected approximately 420 bp, but the other species-specific primers failed to amplify any products (Figure. 2).

This is the first report of the detection of *M. arenaria* on lemon balm grown as a medicinal and aromatic plant in Türkiye. The damage and prevalence of plant parasitic nematodes has been currently increasing in medicinal and aromatic plants. With these findings, the damage of root-knot nematodes could be eliminated by crop rotation, using non-host plants or resistant cultivars.

Autor's Contributions

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

Akhondzadeh S, Nooroonzian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M 2003. *Melissa officinalis* extract in the treatment of patient with mild to moderate Alzheimer's disease: A double blind, randomised, placebo-controlled trial. Journal of Neurol Neurosurg Psychiatry 6(4): 625-632.

Ataş H, Uysal G, Gözel Ç, Özalp T, Gözel U, Devran Z 2021 First report of root-knot nematode, *Meloidogyne incognita* on calendula in Turkey. Journal of Nematology 53: 1-5.

Baytop T 1994. Türkçe Bitki Adları Sözlüğü. Atatürk Kültür, Dil ve Tarih Yüksek Kurumu. Türk Dil Kurumu Yayınları, Türk Tarih Kurumu Basımevi, Ankara, 578 sy.

Bokor P, Tancik J, Haban M, Marinkovic BJ, Polacek M 2008. The occurrence of pests [and diseases] on lemon balm (*Melissa* [ie Melissa] *officinalis*) and garden sage (*Salvia officinalis*). Matica Srpska Proceedings for Natural Sciences.

Chitwood BG 1949. 'Root-knot nematodes' Part 1. A revision of the genus *Meloidogyne* Goeldi, 1987. Proceedings of the Helminthological Society of Washington 16(2): 90-104.

Carneiro RMDG, Carneiro RG, Abrantes IDO, Santos MDA, Almeida MRA 1996. *Meloidogyne paranaensis* n. sp. (Nemata: Meloidogynidae), a root-knot nematode parasitizing coffee in Brazil. Journal of Nematology 28(2): 177-189.

Cliff GM, Hirschmann H 1985. Evaluation of morphological variability in *Meloidogyne arenaria*. Journal of Nematology 17(4): 445.

Devran Z, Polat İ, Mıstanoğlu İ, Baysal Ö 2018. A novel multiplex PCR tool for simultaneous detection of three root-knot nematodes. Australasian Plant Pathology 47: 389-92.

Hooper DJ 1986. Extraction of free-living stages from soil. (Laboratory Methods for Work with Plant Soil Nematodes, Her Majesty's Stationary Office, London: Ed. Southey JF) 5-30.

Jepson SB 1987. Identification of Root-knot Nematodes *Meloidogyne* species. CABI Wallingford, Oxfordshire.

Karanastasi E, Conceição ID, Santos MD, Tzortzakakis E, Abrantes IDO 2008. Occurrence of the root-knot nematode *Meloidogyne arenaria* on balm and in a mixed population with *M. javanica* on grapevine in Greece. Helminthologia 45(1): 52-53.

Karssen G 2002. The plant parasitic nematode genus *Meloidogyne* Goeldi, 1892 (Tylenchida) in Europe. Leiden: Brill Academic Publishers.

Karssen G, Wesemael W, Moens M 2013. Root-knot nematodes. (Plant Nematology, CAB International, Wallingford: Ed. Perry RN, Moens M) 59-90.

Kindelan A, Gutierrez CE, Martinez M 1990. New pathogens in *Melissa officinalis*. Revista Plantas Medicinales 10: 83-85.

Koshy PK, Pandey R, Eapen SJ 2005. Nematode Parasites of Spices, Condiments and Medicinal Plants. (*Plant* Parasitic Nematodes in Subtropical and Tropical Agriculture, University of Bonn, Germany, J. Bridge, CABI, Bioscience, Egham, Surrey, U.K: Ed. Luc M, Orstom F, Sikora RA) 751-791.

Mendonça CI, Mattos JKA, Carneiro RMDG 2017. Host status of medicinal plants to *Meloidogyne paranaensis*. Nematropica 47: 49-54.

Mill RR 1982. *Melissa* L. (Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh: Ed. Davis PH) vol 7.

Pandey R 2017. Diseases of medicinal and aromatic plants: Insights in nematode bio management. Indian Phytopathology 70(1): 12-21.

Randig O, Bongiovanni M, Carneiro RM, 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.

Seinhorst JW 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 67-69.

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.

Verma PS, Singh A, Rahaman L, Bahl JR 2015. Lemon balm (*Melissa officinalis* L.) an herbal medicinal plant with broad therapeutic uses and cultivation practices: A review. Internal Journal of Recent Advances in Multidisciplinary Research 2: 928-33.

Walker JT 1995. Garden herbs as hosts for southern root-knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood, race 3]. HortScience 30(2): 292-293.

Whitehead AG 1968. Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four new species. The Transactions of Zoological Society of London 31(3): 263-401.

Wishart J, Phillips MS, Blok VC 2002. Ribosomal Intergenic Spacer: A Polymerase chain reaction diagnostic for *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla*. Nematology 92: 884-92.

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 2: 847-853.