Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Determination of plant parasitic nematodes on some oil rose growing areas of Isparta province in Türkiye

Türkiye'de Isparta ili yağ gülü yetiştirilen bazı alanlarında bitki paraziti nematodların belirlenmesi

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ARTICLE INFO

Article history:

DOI: 10.16955/bitkorb.1244955

Received : 30-01-2023

Accepted : 14-03-2023

Keywords:

oil rose, Rosaceae, root lesion nematode, root-knot nematode

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ABSTRACT

The study was carried out to investigate plant parasitic nematodes in oil rose growing areas in Isparta province in July-August 2022. Soil samples were collected from 4 districts and the study was carried out with 65 samples. Plant parasitic nematodes were extracted by the modified Baermenn funnel technique. A total of 12 genera of plant parasitic nematodes were determined in the study. These are *Tylenchus* spp., *Aphelenchus* spp., *Pratylenchus* spp., *Ditylenchus* spp., *Dorylaimus* spp., *Paratylenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., and *Merlinius* spp. The *Pratylenchus* (52.3%), *Paratylenchus* (38.5%), and *Helicotylenchus* (41.5%) seemed to be the most prevailing genera. The least common genus is *Meloidogyne* (7.6%). *Meloidogyne* species were identified molecularly by using species-specific primers from second-stage juveniles. The 5 samples taken from cultivated oil rose fields were infected and detected with *Meloidogyne* hapla Chitwood, 1949. Densities of *M. hapla* varied between 60-480/100 g soil. This is the first report of *M. hapla* on oil rose (*Rosa damascena* Mill.) in Türkiye.

INTRODUCTION

Rosa damascena Mill. (Rosaceae) is an important essential oil plant. Bulgaria and Türkiye are the largest rose processing countries in the world. Approximately 15.000 tons of rose flowers are produced annually worldwide; about 8.500 tons of this production is made in Türkiye (Ersan and Başayiğit 2022). Rose oil is a very valuable product used in the perfumery, cosmetics, food industry, and pharmacy. Türkiye produces more than half of the world's rose oil and 44.4% of the world's rose concrete (Izgi 2022). 81.8% of Türkiye's oil rose production is made in Isparta is famous for its rose oil, on the other hand, it also has a privileged position due to profit per unit area, employment opportunities, and exportation. About 84% of rose production in Isparta is provided by Gönen, Keçiborlu, and Merkez districts (Arıcı et al. 2022, Gul et al. 2015). Approximately 5 tons of concrete, 2 tons of absolute, and 1.5 tons of rose oil are produced annually in 20 distillation and extraction facilities operating in Isparta province. Over 15 million Euros of foreign currency enters the economy of Isparta annually from the export of these products (Arıcı et al. 2022, Baydar 2016).

Many nematode species are agricultural pests that cause large yield losses (Stirling and Stirling 2003). Plant parasitic nematodes infect plants and caused plant nutrient deficiency and may develop symptoms such as root galls, lesions, excessive

branching, blunt root formation, or root rot (Agrios 1997, Ogallo et al. 1997). In addition, plant parasitic nematodes can cause the sensitivity in the plant to secondary microorganisms (Göze Özdemir et al. 2022). Rose plants are suspected to be infected with numerous plant parasitic nematodes, *Meloidogyne* spp., and *Pratylenchus* spp. are stated as economically important parasites of rose varieties (Fox 2001). *Pratylenchus* spp. migrate and feed within the roots, resulting in lesions initially appearing as spots along the root surface. Later, these areas may coalesce to become large areas of necrotic tissue (Castillo and Vovlas 2007). *Meloidogyne* spp. are sedentary endoparasites with a broad host range, highly pathogenic, and can destroy the host resistance (Jones et al. 2013). *Meloidogyne* spp. affect the supply of water and nutrients to plants, thereby adversely affecting growth (Portillo et al. 2013). Root-knot nematodes have been reported in rose-growing areas around the world (Oloo et al. 2009, Wang et al. 1999). Growers in commercial hydroponic rose culture in the Netherlands reported up to 40% production losses because of root-knot nematodes (García Victoria and Amsing 2005). *Rosa indica* L. and *Rosa multiflora* Thunb. are frequently attacked by *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae) (Wang et al. 2004). While *Meloidogyne hapla* may reproduce either by meiotic parthenogenesis or by *amphimixis*, *M. incognita*, *M. javanica*, and *M. arenaria* reproduce by obligate mitotic parthenogenesis (Triantaphyllou 1985).

To increase the yield and quality of roses, first of all, pests and diseases must be identified and controlled in the growing areas. Akgül and Ökten (1997) reported that 22 species of Tylenchida were determined in oil rose areas of Isparta province. However, it has been observed that no study has been done recently. Therefore, this study aimed to determine the plant parasitic nematodes in oil roses in Isparta province between June and August 2022.

MATERIALS AND METHODS

The main material of the research consists of soil samples taken from the areas where rose production is made in Isparta and plant parasitic nematodes obtained.

Table 1. Sampling locations and numbers in the oil rose fields of Isparta province, Türkiye

District	Village/Town	Sampling number (Soil sampling)
Atabey	İslamköy	5
	Harmanören	5
	Merkez	3
Gönen	Güneykent	10
	Gümüşgün	5
	İğdecik	4
Keçiborlu	Merkez	2
	Kılıç	6
	Senir	9
Central	Merkez	2
	Aliköy	2
	Deregümü	3
	Kayıköy	2
	Yakaören	5
	Gelincik	2

Sampling was made at different time intervals between June and August of 2022. A total of 65 samples were taken from 15 different areas in Isparta. Sampling locations and sample numbers are shown in Table 1. Soil samples were taken from a depth of 0-30 cm with a shovel to represent the field from different points of the field, which showed stunting and yellowness in the oil rose. Approximately 1 kg of soil samples were taken from each field, placed in polyethylene bags, labeled, and brought to the laboratory in an ice box.

Nematode identification

Plant parasitic nematodes were extracted from 100 g of dry soil from each sample using a modified Baermann funnel technique (Hooper 1986, Whitehead and Hemming 1965). Nematodes were counted according to genera under the light microscope at 20x magnification.

Molecular identification

DNA isolation from root-knot nematode larvae obtained from soil samples was carried out using the "DNAeasy Tissue and Blood Kit" (Qiagen, Hilden, Germany). Species-specific PCR primers were used for molecular identification which was conducted by thermocycler (Veriti Thermal Cycler, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) (Table 2). PCR amplifications were performed in

Table 2. Species-specific primers of root-knot nematodes for molecular identification

Species	Name of primer	Primer sequences (5-3)	Fragment (bp)	Reference
M. arenaria	FAR	TCGGCGATAGAGGTAAATGAC	420	Zijlstra et al. 2000
	RAR	TCGGCGATAGACACTACAACT		
M. javanica	FJAV	GGTGCGCGATTGAACTGAGC	670	Zijlstra et al. 2000
	RJAV	CAGGCCCTTCAGTGGAACTATAC		
M. incognita	INCK14R	CCCGCTACACCCTCAACTTC	399	Randig et al. 2002
	INCK14F	GGGATGTGTAAATGCTCCTG		
M. hapla	JMVhapla	GGATGGCGTGCTTTCAAC	440	Wishart et al. 2002
	JMV1	TTTCCCCTTATGATGTTTACCC		
	JMV2	AAAAATCCCCTCGAAAAATCCACC		

a total volume of 25 µl reaction mixtures, each containing 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 products were separated using agarose electrophoresis in 2% gel (Agarose Type I, Sigma-Aldrich, St. Louis, MO, USA) staining with ethidium bromide, then visualized and photographed under UV light using a gel documentation system.

Plant parasitic nematode community analyses

The occurrence, absolute, and relative frequency of plant parasitic nematodes in the study of the genus in oil rose areas in Isparta province were calculated using the formulas below (Evlice 2021, İmren 2018, Norton 1978).

$$\text{The prevalence rate of genus} = \frac{\text{Number of infected samples in district}}{\text{Total number of samples surveyed}} \text{X } 100$$

$$\text{Absolute frequency} = \frac{\text{Number of samples containing a genus}}{\text{Number of samples collected}} \text{X } 100$$

$$\text{Relative frequency} = \frac{\text{Relative frequency}}{\text{Sum of frequency of all genus}} \text{X } 100$$

RESULTS

It was determined that there are 12 genera of plant parasitic nematodes where oil rose is produced in Isparta province. In the study, *Tylenchus* spp., *Aphelenchus* spp., *Pratylenchus* spp., *Ditylenchus* spp., *Dorylaimus* spp., *Paratylenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., and *Merlinius* spp. were detected. The prevalence rate of presence is shown in Table 3. The highest prevalence was found in the genus *Pratylenchus* with 52.3%, while *Helicotylenchus* spp. was ranked second with 41.5%. The least common genus was *Meloidogyne* (7.6%), and it was detected in only 5 areas. The prevalence of *Longidorus* spp. and *Xiphinema* spp. were determined as 10.8% and 9.2%, respectively (Table 3).

Table 3. The prevalence rate of plant parasitic nematodes in oil rose areas in Isparta province

Plant parasitic nematode	% Prevalence
Pratylenchus spp.	52.3
Helicotylenchus spp.	41.5
Paratylenchus spp.	38.5
Aphelenchus spp.	24.6
Tylenchus spp.	20
Ditylenchus spp.	20
Merlinius spp.	20
Dorylaimus spp.	13.8
Tylenchorhynchus spp.	10.8
Longidorus spp.	10.8
Xiphinema spp.	9.2
Meloidogyne spp.	7.6

The absolute and relative frequency at the genus level in the districts in Isparta is given in Table 4. The absolute frequency was recorded in *Meloidogyne* spp. with 15.3% in Atabey (İslamköy), 9.5% in Gönen (Güneykent), and 5.8% (Senir) in Keçiborlu district. *Meloidogyne* spp. was not found in the samples taken from the villages of the central district of Isparta. The lowest relative frequency in the Atabey district was also found in *Meloidogyne* spp. and *Xiphinema* spp. with 5.6%. *Paratylenchus* spp. (53.8%) was determined as the most common genus after *Pratylenchus* spp. (69.2%) and *Helicotylenchus* spp. (61.5%). in the Atabey district. While the lowest relative frequency in Gönen district was also found in *Longidorus* spp. and *Tylenchorhynchus* spp. with 1.7%, the highest was recorded in *Tylenchus* spp. (15.9%), *Pratylenchus* spp. (14.1%) and *Helicotylenchus* spp. (14.1%). In the Keçiborlu district, the highest absolute and relative frequency was determined in *Helicotylenchus* spp. with 41.1% and 15.2%, respectively. *Tylenchorhynchus* spp. was not found in central samples. The relative frequency of *Longidorus* spp. (2.6%) and *Tylenchus* spp. (2.6%) in the central was recorded as the lowest whereas the highest was in *Pratylenchus* spp. with 31.8% (Table 4).

As a result of the molecular identification, *Meloidogyne hapla* Chitwood, 1949 was identified in the five samples (Figure 1). The densities of Güneykent2 and İslamköy1 samples were found to be 480 larvae/100 g soil and 320 larvae/100 g soil, respectively (Figure 2).

Figure 1. PCR products amplified using primers JMV/JMV1/JMV2 (S1:Senir, IS1: İslamköy1, IS2: İslamköy2, G1: Güneykent1, G2: Güneykent2)

Table 4. Population analyses of plant parasitic nematodes on oil rose in Isparta

Plant parasitic nematode	Atabey		Gönen		Keçiborlu		Central	
	AF[1]	RF	AF	RF	AF	RF	AF	RF
Tylenchus spp.	30.7	11.4	42.8	15.9	17.6	6.5	7.1	2.6
Aphelenchus spp.	38.4	14.3	14.2	5.2	23.5	8.7	56.0	21.1
Pratylenchus spp.	69.2	25.7	38.0	14.1	29.4	10.9	85.7	31.8
Ditylenchus spp.	30.7	11.4	9.5	3.5	23.5	8.7	28.5	10.5
Dorylaimus spp.	23.1	8.5	9.5	3.5	11.7	4.3	14.2	5.2
Paratylenchus spp.	53.8	19.9	33.3	12.3	35.2	13.0	21.4	7.9
Longidorus spp.	23.1	8.5	4.7	1.7	11.7	4.3	14.2	5.2
Xiphinema spp.	15.3	5.6	9.5	3.5	11.7	4.3	7.1	2.6
Meloidogyne spp.	15.3	5.6	9.5	3.5	5.8	2.1	0	0
Helicotylenchus spp.	61.5	22.8	38.0	14.1	41.1	15.2	28.5	10.5
Tylenchorhynchus spp.	23.0	8.5	4.7	1.7	17.6	6.5	0	0
Merlinius spp.	30.7	11.4	14.2	5.2	17.6	6.5	21.4	7.9

1 AF: Absolute Frequency, RF: Relative Frequency

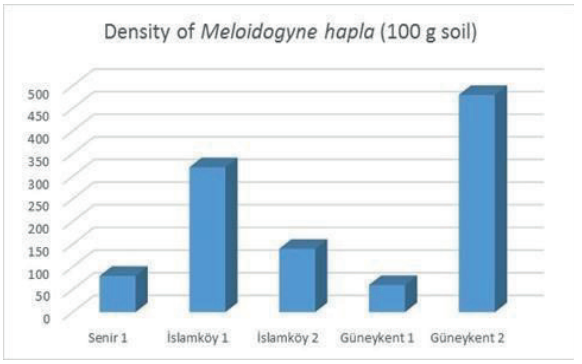

Figure 2. Soil density of *Meloidogyne hapla* in oil rose areas in Isparta province

DISCUSSION

In the present study, the 12 genera belonging to plant parasitic nematodes were identified in oil rose areas of Isparta, Türkiye. *Pratylenchus* spp. and *Helicotylenchus* spp. was determined as major genus and *Meloidogyne* spp. was found as a minor genus in the study. In a previous study, 22 species of Tylenchida were determined and three species (*Pratylenchus neglectus* (Rensch, 1924) Filipjev and Schuurmans-Stekhoven, *Ditylenchus clarus* Thorne and Malek, 1968 and *Filenchus plattensis* Thorne and Malek, 1968) were reported as the most common species in the rose growing areas of Isparta (Akgül and Ökten 1997). However, *Filenchus* spp. was not found in this study. In Florida, at least 30 species of plant parasitic nematodes have been recovered from the soil around rose roots (Lehman 1982). Nour El-Deen et al. (2015) reported the genera *Meloidogyne*, *Rotylenchulus*, *Xiphinema*, and *Pratylenchus* in their study on Taify rose planted areas in Saudi Arabia. The percentage of these nematodes was found 30%, 29.3%, 16.5%, and 11.3%, respectively. However, in this study, *Rotylenchus* spp. was not detected. Red raspberry is in the Rosaceae family like rose, Mokrini et al. (2019) reported that the most common plant-parasitic nematodes (PPN) were *Pratylenchus* spp., *Meloidogyne* spp. and *Helicotylenchus* spp. in raspberry in Morocco's Souss-Massa region. *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Criconemoides xenoplax*, and *Ditylenchus dipsaci* were associated with red raspberry disease (Kroese et al. 2016, Poiras et al. 2014). In a study conducted by Magnusson and Tangvik (2018) in Norway, in raspberry (*Rubus idaeus* L.) orchards, *Tylenchus davainei* Bastian, 1865, *Cephalenchus leptus* Siddiqi, 1963, *Tylenchorhynchus dubius* (Buetschli, 1873) Filipjev, 1936, *Pratylenchus crenatus* Loof, 1960, *Pratylenchus penetrans* (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941, *Pratylenchus fallax* Seinhorst 1968, *Helicotylenchus canadiensis* Wassem, 1961, *Helicotylenchus variocaudatus* Yuen, 1964, *Rotylenchus fallorobustus* Sher, 1965, *Paratrichodorus pachydermus* Seinhorst, 1954 and *Longidorus elongatus* (de Man, 1876) Micoletzky, 1922 species were identified. It has been reported that one of the biggest threats to the production of red raspberries is the root lesion nematode *P. penetrans* (Rudolph et al. 2017, Zasada and Moore 2014, Zasada and Walters 2016). *Raspberry* (*R. idaeus*) and blackberry (*Rubus fruticosus* L.) orchards in Türkiye, a total of 34 species, including 18 genera, were identified and the most encountered species were found *P. penetrans*, *P. neglectus*, *Filenchus filiformis* Bütschli, 1873, *Filenchus anguilonius* (Wu, 1969) Lownsberry & Lownsbery, 1985, *Helicotylenchus digonicus* Perry, 1959 and *Aphelenchus avenae* Bastian, 1865 (Çalışkan 2019).

In the present study, it was determined that Atabey (IS1, IS2), Gönen (G1, G2), and Keçiborlu (S1) district was infected with root-knot nematode, *M. hapla*. This is the first report of *M. hapla* on oil rose in Türkiye. *Meloidogyne hapla* was described first time on *Solanum tuberosum* from the USA by Chitwood (1949) (Evans et al. 1993). This specie has a wide host range, which is more common in temperate

regions and affects more than 550 crops and weed species (Grandison 1983, Jepson 1987). In addition, *M. hapla* eggs and juveniles can survive field temperatures below 0 °C (Karssen et al. 2013). Unlike thermophilic species such as *M. arenaria*, *M. incognita*, and *M. javanica*, which often cause large, coalesced galls that can cover the entire root system, the galls formed by *M. hapla* are usually smaller and more discrete (Nyoike et al. 2012). It was determined on pepino, kiwifruit, tomatoes, pepper, potatoes, strawberry, and eggplant in Türkiye (Akyazi et al. 2012, 2017, Evlice et al. 2022, Özarslandan et al. 2010, 2021, Uysal et al. 2017). It is considered that *Meloidogyne hapla* is one of the main plant parasitic nematode specie restricting rose cultivation (*Rosaceae* spp.) and is reported to have a worldwide distribution (Fox 2001, Meressa et al. 2014, Oloo et al. 2009, Wang et al. 2004). *Meloidogyne hapla* and *P. penetrans* are the most widespread nematodes in strawberries in the Rosaceae worldwide (Nyoike et al. 2012, Samaliev and Mohamedova 2011). In addition, Göze Özdemir (2022) detected *M. incognita* and *M. arenaria* infestation in lavender fields in Isparta province.

To the author's knowledge, *Pratylenchus* spp. was detected as a widespread nematode in an oil rose field in Isparta, Türkiye. Finding the northern nematode, *M. hapla* is important in Isparta. This nematode is a polyphagous and quarantine pest. However, there is no information about the effect of the amount of essential oil. Thus, it is important to determine the effect of the amount of essential oil in the continuation of the study. In addition, resistant cultivars against *M. hapla* should be determined by doing reaction studies with different rootstocks and cultivars.

ÖZET

Çalışma, Isparta ilinde yağ gülü yetiştirilen alanlarda bitki paraziti nematodların araştırılması amacıyla Temmuz-Ağustos 2022 tarihlerinde gerçekleştirilmiştir. Dört ilçeden toprak örneği alınmıştır ve çalışma 65 örnek ile yürütülmüştür. Çalışmada toplam 12 cinse ait bitki paraziti nematodlar tespit edilmiştir. Bunlar *Tylenchus* spp., *Aphelenchus* spp., *Pratylenchus* spp., *Ditylenchus* spp., *Dorylaimus* spp., *Paratylenchus* spp., *Longidorus* spp., *Xiphinema* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp. ve *Merlinius* spp.'dir. *Pratylenchus* (%52.3), *Paratylenchus* (%38.5) ve *Helicotylenchus* (%41.5) en yaygın cinsler olarak görülmüştür. En az yaygın olan cins ise *Meloidogyne*'dir (%7.6). Kök ur nematod türlerinin moleküler tanımlaması, larvalardan türe özgü primerler ile belirlenmiştir. Yağ gülü ekimi yapılan tarlalardan alınan 5 örnekte bulaşıklık saptanmış ve *Meloidogyne* hapla Chitwood, 1949 tespit edilmiştir. *Meloidogyne hapla*'nın yoğunlukları 60-480/100 g toprak arasında değişmektedir. Bu, *M. hapla*'nın Türkiye'de yağ gülü (*Rosa damascena* Mill.) üzerinde tespit edildiği ilk rapordur.

Anahtar kelimeler: yağlık gül, Rosaceae, kök lezyon nematodu, kök ur nematodu

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Cite this article: Göze Özdemir, F. G. (2023). Determination of plant parasitic nematodes on some oil rose growing areas of Isparta province in Türkiye. Plant Protection Bulletin, 63-2. DOI: 10.16955/bitkorb.1244955

Atıf için: Göze Özdemir, F. G. (2023). Türkiye'de Isparta ili yağ gülü yetiştirilen bazı alanlarında bitki paraziti nematodların belirlenmesi. Bitki Koruma Bülteni, 63-2. DOI: 10.16955/bitkorb.1244955