Türk. entomol. derg., 2022, 46 (2): 187-197
DOI: http://dx.doi.org/10.16970/entoted.1092654

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Prevalence of root-knot nematodes and their effects on fruit yield in kiwifruit orchards in Samsun Province (Türkiye)[1]

Samsun İli (Türkiye) kivi bahçelerindeki kök-ur nematodlarının yaygınlığı ve meyve verimine etkileri

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Abstract

The aims of this study were to determine the distribution of root-knot nematodes (*Meloidogyne* spp.) in kiwifruit orchards in Samsun Province (Türkiye) and assess the effect of *Meloidogyne* spp. on fruit yields in naturally infested orchards. The survey was conducted in 25 kiwifruit orchards in September-November 2017. In addition, the data on fruit yields was obtained in two orchards at the harvest time in 2018. Fifty-six soil and root samples were collected from infested orchards. Species identification was performed by esterase phenotype and PCR with species-specific primers. *Meloidogyne* spp. were found in 92% of the orchards surveyed. *Meloidogyne luci* (Carneiro et al., 2014) (Tylenchida: Meloidogynidae) was detected in 59% of the samples, followed by *Meloidogyne hapla* (Chitwood, 1949) in 41%, *Meloidogyne arenaria* (Neal, 1889) in 27% and *Meloidogyne incognita* (Kofoid & White, 1919) in 2%. Regarding the distribution of *Meloidogyne* spp. in kiwifruit orchards, *M. luci* was found in 74%, *M. hapla* in 57%, *M. arenaria* in 39% and *M. incognita* in 4% of orchards infested. *Meloidogyne luci* was found for the first time in the kiwifruit orchards of Türkiye in this study. It was also determined that *Meloidogyne* spp. caused significant yield losses in kiwifruit orchards, and yield losses of 36 and 49% were detected in two orchards infested with *Meloidogyne* spp., respectively.

Keywords: *Actinidia deliciosa*, distribution, identification, *Meloidogyne*, yield

Öz

Bu çalışmanın amacı, Samsun İli (Türkiye) kivi bahçelerinde kök-ur nematodlarının (*Meloidogyne* spp.) dağılımının belirlenmesi ve doğal olarak bulaşık bahçelerde meyve verimi üzerine *Meloidogyne* spp.'nin etkisinin değerlendirilmesidir. Sürvey 2017 yılı Eylül-Kasım aylarında 25 kivi bahçesinde yürütülmüştür. Ayrıca, 2018 yılı hasat zamanında iki bahçede meyve verimlerine ilişkin veriler elde edilmiştir. Bulaşık bahçelerden 56 toprak ve kök örneği alınmıştır. Tür teşhisleri esteraz fenotipi ve türe özgü primerler ile yapılan PCR ile gerçekleştirilmiştir. *Meloidogyne* spp., sürvey yapılan bahçelerin %92'sinde bulunmuştur. Örneklerin %59'unda *Meloidogyne luci* (Carneiro et al., 2014) (Tylenchida: Meloidogynidae) tespit edilmiş, bunu %41 ile *Meloidogyne hapla* (Chitwood, 1949), %27 ile *Meloidogyne arenaria* (Neal, 1889) ve %2 ile *Meloidogyne incognita* (Kofoid & White, 1919) izlemiştir. *Meloidogyne* spp.'nin kivi bahçelerindeki dağılımı ile ilgili olarak, bulaşık bahçelerin %74'ünde *M. luci*, %57'sinde *M. hapla*, %39'unda *M. arenaria* ve %4'ünde *M. incognita* bulunmuştur. *Meloidogyne luci*, Türkiye'deki kivi bahçelerinde ilk kez bu çalışmada bulunmuştur. Ayrıca, *Meloidogyne* spp.'nin kivi bahçelerinde önemli verim kayıplarına neden olduğu ve *Meloidogyne* spp. ile bulaşık iki bahçede sırasıyla %36 ve %49 verim kaybı olduğu belirlenmiştir.

Anahtar sözcükler: *Actinidia deliciosa*, dağılım, teşhis, *Meloidogyne*, verim

[1] This study was partly presented as a poster at the 33th Symposium of the European Society of Nematologists (9-13 September 2018, Ghent, Belgium) and published as an abstract in the abstract book.

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Received (Alınış): 24.03.2022 Accepted (Kabul ediliş): 14.06.2022 Published Online (Çevrimiçi Yayın Tarihi): 18.06.2022

Introduction

Actinidia chinensis Planch. and *Actinidia deliciosa* (A. Chev.) C.F.Liang et A.R.Ferguson, which are closely related species, are cultivated for commercial kiwifruit production globally, with most of the production from the latter as in Türkiye (Ferguson & Seal, 2008; Atak, 2015). Kiwifruit has a significant nutritional content that is beneficial to human health. Particularly, a high concentration of vitamin C and a variety of other nutrients, including vitamins, fiber, and antioxidants in its nutrient content have been linked to digestive, immune, and metabolic health advantages (Richardson et al., 2018).

World kiwifruit production was about 4.4 Mt in 2020 (FAO, 2022). China was largest producer with 50% of global production (2.2 Mt), followed by New Zealand (625 kt), Italy (522 kt), Greece (307 kt), Iran (290 kt) and Chile (159 kt). Türkiye ranked seventh with an annual production of 74 kt (FAO, 2022). The kiwifruit cultivation in Türkiye began at the Atatürk Central Horticultural Research Institute in Yalova in 1988 (Atak, 2015). The plantation area has rapidly expanded, which almost doubled in the last decade, from 1,720 ha in 2010 to 3,260 ha in 2020 (TUIK, 2022). In 2021, the average yield was 45 kg per fruiting vine (TUIK, 2022). The Black Sea Region is the second-largest regional production area with 40% of kiwifruit plantations of Türkiye, and Samsun, Ordu and Rize Provinces are the primary producers in this region. In recent years, interest in kiwifruit production has increased as an alternative to hazelnut in Samsun Province.

Root-knot nematodes (RKN), *Meloidogyne* spp., are among the potential threats to limiting kiwifruit production worldwide. Their obligate sedentary parasitism in plant roots alters the physiological processes of plants that can result in the reduction quantity and quality of yield. Di Vito et al. (1988) reported that *Meloidogyne incognita* (Kofoid & White, 1919) (Tylenchida: Meloidogynidae) caused a substantial reduction in the relative aerial growth of plants in pot experiments in a greenhouse. El-Borai & Duncan (2005) reported that RKN infestations in kiwifruit orchards of Italy and France were associated with poor plants. It has been estimated that RKN caused up to 40% yield losses in some kiwifruit orchards of China (as cited in Tao et al., 2017). *Meloidogyne incognita* and *Meloidogyne hapla* (Chitwood, 1949) have been the most commonly encountered species among eight species of RKN (*Meloidogyne aberrans* Tao et al., 2017, *Meloidogyne actinidiae* Li & Yu, 1991, *Meloidogyne arenaria* Neal, 1889, *Meloidogyne ethiopica* Whitehead, 1968, *M. hapla, M. incognita*, *Meloidogyne javanica* Treub, 1885, *Meloidogyne luci* Carneiro et al., 2014) found associated with kiwifruit in different countries of the world (Scotto La Massese, 1973; Vovlas & Roca, 1976; Haygood et al., 1990; Watson et al., 1992; Castillo et al., 1993; Philippi et al., 1996; Maafi & Mahdavian, 1997; Waliullah, 2005; Carneiro et al., 2007; Ma et al., 2007; Somavilla et al., 2011; Tzortzakakis et al., 2011; Conceição et al., 2012; Akyazi et al., 2017; Tao et al., 2017; Shokoohi & Mashela, 2020). Similarly, these two species were found to commonly occur in Türkiye when a survey of RKN in kiwifruit orchards was conducted in a few provinces (Akyazı & Felek, 2013; Akyazi et al., 2017; Evlice & Özdemir, 2021). In addition to these two species, *M. arenaria* was found only in a kiwifruit orchard in Ordu Province (Akyazi et al., 2017).

Previous surveys revealed the widespread distribution of RKN in vegetable-growing areas in Samsun Province of Türkiye (Katı & Mennan, 2006; Aydınlı & Mennan, 2016; Aydınlı, 2018). Particularly, *M. luci* was commonly found (Aydınlı & Mennan, 2016; Aydınlı, 2018). The esterase phenotype (M3) of one population from Türkiye reported as an unidentified RKN population by Esbenshade & Triantaphyllou (1985) is very similar to that of *M. luci* (L3). Thus, this nematode species may have been present in Türkiye for many years. We hypothesized that this species might occur in kiwifruit orchards in Samsun Province. Therefore, the first objective of this study was to identify the RKN species in kiwifruit roots. Despite a few studies indicating the effect of *Meloidogyne* on the plant growth in kiwifruit (Di Vito et al., 1988; Philippi & Budge, 1992), to date, there are no studies on the effect of RKN on fruit yield. Therefore, the second objective of this study was to determine the relative fruit yield losses in kiwifruit vines naturally infested with RKN compared to non-infested ones.

Materials and Methods

Survey

The survey was conducted in September-November 2017 in 25 kiwifruit orchards in six districts (Atakum, Bafra, Çarşamba, Ondokuzmayıs, Salıpazarı and Terme) of Samsun Province, Türkiye. The roots of 10-20 plants from each kiwifruit orchard were examined for galls caused by root-knot nematode. When kiwifruit roots with galls were detected, roots and rhizosphere soils were collected. According to the size of the kiwifruit orchard, the sampling area in each orchard was divided into two to four subsections. In each subsection, roots and soils from three to five kiwifruit plants were placed together into a labeled polyethylene bag to obtain a composite sample and transferred to the Nematology Laboratory at Ondokuz Mayıs University, Samsun. A total of 56 samples were processed within 3 days.

Nematode extraction and identification

Egg masses and females were collected randomly from infested kiwifruit roots using forceps, which was sterilized between samples to prevent cross-contamination. Five to 10 egg masses per sample were packed in an Eppendorf tube and placed at -20°C until DNA extraction. Young egg-laying females were macerated with a pestle in a tube with 5 µL of extraction buffer (20% sucrose with 1% Triton X-100), then the specimens were immediately placed in a freezer at -20°C. Females and egg masses were used for biochemical (esterase phenotype, EST) and molecular (PCR with species-specific primers) identification of samples, respectively. In some samples, there were unsuitable females for the esterase study. Thus, soil samples were placed into 600 mL pots, and individual tomato (*Solanum lycopersicum* L. cv. Falcon) seedlings were planted singly. The tomato plants were maintained in a greenhouse at 22-30°C. After 6-8 weeks, young egg-laying females were handpicked and prepared as described above. For each sample, at least 20 females collected from roots of kiwifruit and tomato were analyzed for their esterase phenotypes. Electrophoresis was performed according to Aydınlı & Mennan (2016). Following electrophoresis, the gels were removed from the glass plates and stained for esterase activity. Females from pure isolate of *M. javanica* were included in each gel as a reference.

Total DNA was extracted from the egg masses for molecular identification using the DNeasy Tissue & Blood Kit (QIAGEN, Hilden, Germany). PCRs were performed with specific primers, namely Far/Rar (Zijlstra et al., 2000) for *M. arenaria*, inc-K14F/R (Randig et al., 2002) for *M. incognita*, JMV1/JMV2/JMVhapla (Wishart et al., 2002) for *M. hapla*, and Me309F/Me549R (Gerič-Stare et al., 2019) for *M. luci*. The PCR mixture (25 µL) contained 12.5 µl of BioMix Red (Bioline), 1 µl of each primer (0.4 µM of each primer), and 5 µl of DNA template. A positive control with reference DNAs and a negative control (water) were included. PCR amplification was done using a T-100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). Samples were amplified using cycling conditions of 94ºC for 3 min, followed by 35 cycles of 94ºC for 30 s, annealing temperature for 30 s and 72ºC for 1 min, and a final cycle of 72ºC for 7 min. Annealing temperatures were 50ºC for primers Me309F/Me549R, 55ºC for primers JMV1/JMV2/JMVhapla, 56ºC for primers Far/Rar, and 60ºC for primers inc-K14F/R. All primer pairs were used for all samples in the study. The products from PCRs were separated by electrophoresis in 2% agarose gel stained with ethidium bromide and visualized under UV light.

Fruit yield

Two commercial kiwifruit (cv. Hayward) orchards in Çarşamba District were selected to evaluate the effect of RKN on fruit yield. The data on fruit yield was obtained at harvest time on 26 October 2018. Orchard A located in Dikbıyık (41°13'13" N, 36°37'17" E) was in a 9-year-old, while orchard B located in Eğrikum (41°08'07" N, 36°43'07" E) was in a 6-year-old kiwifruit plantation. According to survey in this study, both orchards had been previously known naturally infested with mixed *Meloidogyne* populations. Each orchard was initially checked for root-knot galls at harvest time in 2018, and four nematode-infested vines were

determined. The soil samples were collected, and second-stage juveniles (J2) were obtained from one 100 mL soil subsample per vine using the tray extraction method (Whitehead & Hemming, 1965). Four vines having no gall symptoms caused by root-knot nematode were marked as non-infested vines. Fruits were harvested from nematode-infested and non-infested vines, and yields were expressed in kg/vine. Yields were compared within each kiwifruit orchard by t-test at $P \leq 0.05$ significance level using SPSS statistical software to determine the significance of differences between nematode-infested vines and non-infested vines.

Results

RKNs were present in all surveyed districts in Samsun Province (Figure 1). Of the 25 kiwifruit orchards surveyed, 23 (92%) were infested with RKN (Table 1). In 56 samples collected from infested orchards, species identification was done using biochemical (EST) and molecular (PCR with species-specific primers) analysis. In 18 root samples of kiwifruit, females were not suitable for biochemical identification. Thus, EST phenotypes of these samples were obtained on females collected roots of tomatoes cultured in their soil samples. Four EST phenotypes from 56 samples analyzed were identified associated with the four RKN species: *M. arenaria* (A2), *M. hapla* (H1), *M. incognita* (I2) and *M. luci* (L3) (Figure 2). As expected, species-specific primers, Far/Rar, inc-K14F/R, JMV1/JMV2/JMVhapla and Me309F/Me549R, used in this study that produced a single band of 420 bp for *M. arenaria*, 400 bp for *M. incognita*, 440 bp for *M. hapla*, and 240 bp for *M. luci*, respectively (Figure 3). A species-specific EST phenotype detected in all samples, except four samples (namely 11-3, 11-4, 13-2 and 14-2), was consistently associated with each of the *Meloidogyne* spp. identified by PCR with species-specific primers. In three samples (11-3, 11-4 and 13-2), *M. hapla* species-specific EST phenotype H1 was not observed whereas PCR amplifications were positive for JMV1/JMV2/JMVhapla primers that produced a single band of ~440 bp. Conversely, in sample 14-2, *M. arenaria* species-specific phenotype was detected, but the primer Far/Rar did not give an amplification product.

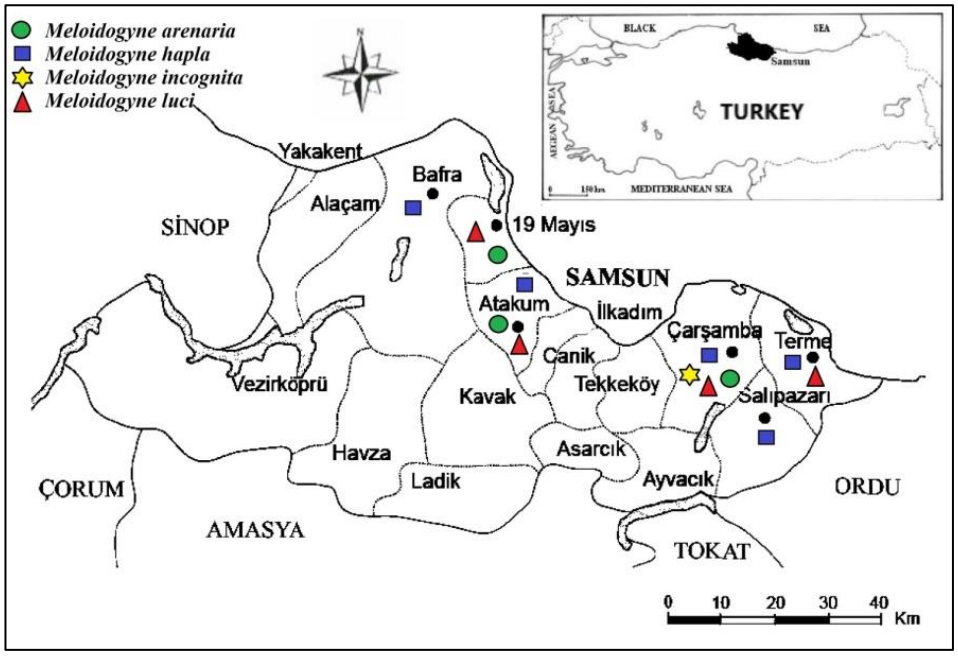

Figure 1. Distribution of *Meloidogyne* spp. in kiwifruit orchards surveyed in Samsun, Türkiye.

Table 1. *Meloidogyne* spp. identified in samples (n = 56) from 23 kiwifruit orchards in Samsun Province, Türkiye

District	Location	Geographic coordinates	Sample no	Esterase phenotype		PCR*	*Meloidogyne* spp.
				Kiwifruit	Tomato		
Atakum	Taflan	41°26'10" N	1-1	L3	L3	Ml	*M. luci*
		36°09'01" E	1-2	H1, L3	L3	Mh, Ml	*M. hapla, M. luci*
		41°26'11" N	2-1	L3	L3	Ml	*M. luci*
		36°09'05" E	2-2	L3	L3	Ml	*M. luci*
		41°26'07" N	3-1	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*
		36°08'43" E	3-2	-	L3	Ml	*M. luci*
		41°24'57" N	4-1	-	L3	Ml	*M. luci*
		36°05'20" E	4-2	-	L3	Ml	*M. luci*
		41°25'07" N	5-1	H1	H1	Mh	*M. hapla*
		36°06'06" E	5-2	H1	H1	Mh	*M. hapla*
		41°26'06" N	6-1	L3	L3	Ml	*M. luci*
		36°09'03" E	6-2	-	L3	Ml	*M. luci*
Bafra	Sürmeli	41°28'52" N	7-1	H1	-	Mh	*M. hapla*
		35°56'32" E	7-2	H1	-	Mh	*M. hapla*
	Darboğaz	41°25'59" N	8-1	H1	-	Mh	*M. hapla*
		35°55'03" E	8-2	H1	H1	Mh	*M. hapla*
Çarşamba	Eğrikum	41°07'41" N	9-1	H1, L3	L3	Mh, Ml	*M. hapla, M. luci*
		36°42'40" E	9-2	L3	L3	Ml	*M. luci*
		41°07'17" N 36°42'57" E	10-1	H1, L3	-	Mh, Ml	*M. hapla, M. luci*
			10-2	H1	-	Mh	*M. hapla*
			10-3	I2	-	Mi	*M. incognita*
		41°08'07" N 36°43'07" E	11-1	A2, L3	A2, L3	Ma, Ml	*M. arenaria, M. luci*
			11-2	H1, L3	L3	Mh, Ml	*M. hapla, M. luci*
			11-3	L3	-	Mh, Ml	*M. hapla, M. luci*
			11-4	L3	L3	Mh, Ml	*M. hapla, M. luci*
	Karamustafalı	41°19'03" N 36°40'46" E	12-1	A2	-	Ma	*M. arenaria*
			12-2	L3	L3	Ml	*M. luci*
			12-3	L3	L3	Ml	*M. luci*
	Dikbıyık	41°13'13" N	13-1	H1	-	Mh	*M. hapla*
		36°37'17" E	13-2	L3	L3	Mh, Ml	*M. hapla, M. luci*
	Yeşilova	41°15'28" N 36°34'05" E	14-1	-	A2	Ma	*M. arenaria*
			14-2	A2, L3	A2, L3	Ml	*M. arenaria, M. luci*
			14-3	A2	A2	Ma	*M. arenaria*
	Demiraslan	41°09'36" N 36°59'02" E	15-1	-	A2, H1	Ma, Mh	*M. arenaria, M. hapla*
			15-2	A2	A2	Ma	*M. arenaria*
			15-3	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*
			15-4	-	A2	Ma	*M. arenaria*
	Beylerce	41°12'25" N 36°40'39" E	16-1	L3	-	Ml	*M. luci*
			16-2	-	L3	Ml	*M. luci*
			16-3	L3	-	Ml	*M. luci*
	Hacılıçay	41°14'20" N	17-1	-	A2	Ma	*M. arenaria*
		36°41'37" E	17-2	-	L3	Ml	*M. luci*
		41°14'48" N 36°41'27" E	18-1	H1	-	Mh	*M. hapla*
			18-2	H1	-	Mh	*M. hapla*
			18-3	-	A2	Ma	*M. arenaria*
		41°14'02" N 36°41'46" E	19-1	-	L3	Ml	*M. luci*
			19-2	L3	L3	Ml	*M. luci*
			19-3	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*

* Ma: *M. arenaria*, Mh: *M. hapla*, Mi: *M. incognita*, Ml: *M. luci*

Table 1. Continued

District	Location	Geographic coordinates	Sample no	Esterase phenotype		PCR*	*Meloidogyne* spp.
				Kiwifruit	Tomato		
Ondokuzmayıs	Engiz	41°29'34" N	20-1	-	A2, L3	Ma, Ml	*M. arenaria, M. luci*
		36°05'44" E	20-2	A2	A2, L3	Ma, Ml	*M. arenaria, M. luci*
Salıpazarı	Yavaşbey	41°06'20" N	21-1	-	H1	Mh	*M. hapla*
		36°50'18" E	21-2	H1	H1	Mh	*M. hapla*
	Kızılay	41°00'23" N	22-1	H1	-	Mh	*M. hapla*
		36°52'11" E	22-2	H1	H1	Mh	*M. hapla*
Terme	Hüseyinmescit	41°09'36" N	23-1	L3, H1	L3	Mh, Ml	*M. hapla, M. luci*
		36°59'02" E	23-2	-	L3	Ml	*M. luci*

* Ma: *M. arenaria*, Mh: *M. hapla*, Mi: *M. incognita*, Ml: *M. luci*

When EST phenotypes and DNA analyses were assessed together, *M. luci* detected in 33 samples (59%) was the most prevalent RKN species. This nematode species was found associated with 18 samples alone and in 15 samples as mixed populations with *M. hapla* (8 samples) or *M. arenaria* (7 samples). *Meloidogyne hapla* was detected in 23 samples (41%), of which one was a mixed population with *M. arenaria*. In 15 samples (27%), *M. arenaria* was found alone (7 samples) or mixed populations with *M. luci* or *M. hapla*. The presence of *M. incognita* was detected only in one sample (2%), having as a single nematode species.

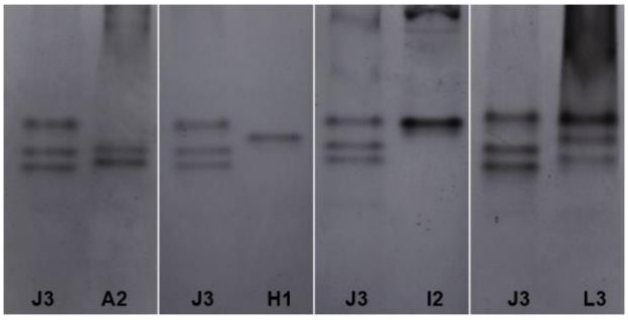

Figure 2. Esterase phenotypes of *Meloidogyne* spp. detected in kiwifruit orchards in Samsun Province, Türkiye (J3, *M. javanica* as reference isolate; A2, *M. arenaria*, H1, *M. hapla*; I2, *M. incognita*; L3, *M. luci*).

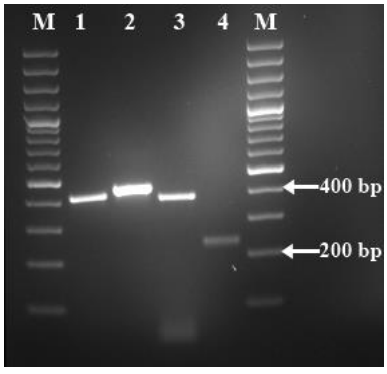

Figure 3. DNA amplification products with *Meloidogyne arenaria*-specific Far/Rar primers (Line 1), *Meloidogyne hapla*-specific JMV primers (Line 2), *Meloidogyne incognita*-specific inc-K14F/R primers (Line 3), *Meloidogyne luci*-specific Me309F/Me549R primers (Line 4) on *Meloidogyne* populations detected in kiwifruit orchards in Samsun, Türkiye (M: molecular marker with 100 bp).

Considering the distribution of *Meloidogyne* spp. in kiwifruit orchards, *M. luci* predominated, being occurred in 74% (17 orchards) of orchards infested, followed by *M. hapla* in 57% (13 orchards), *M. arenaria* in 39% (9 orchards) and *M. incognita* in 4% (1 orchard) (Figure 4). Multiple RKN species, having two or

three species, occurred in 61% (14 orchards) of the orchards infested. Remaining orchards were infested with *M. luci* or *M. hapla*.

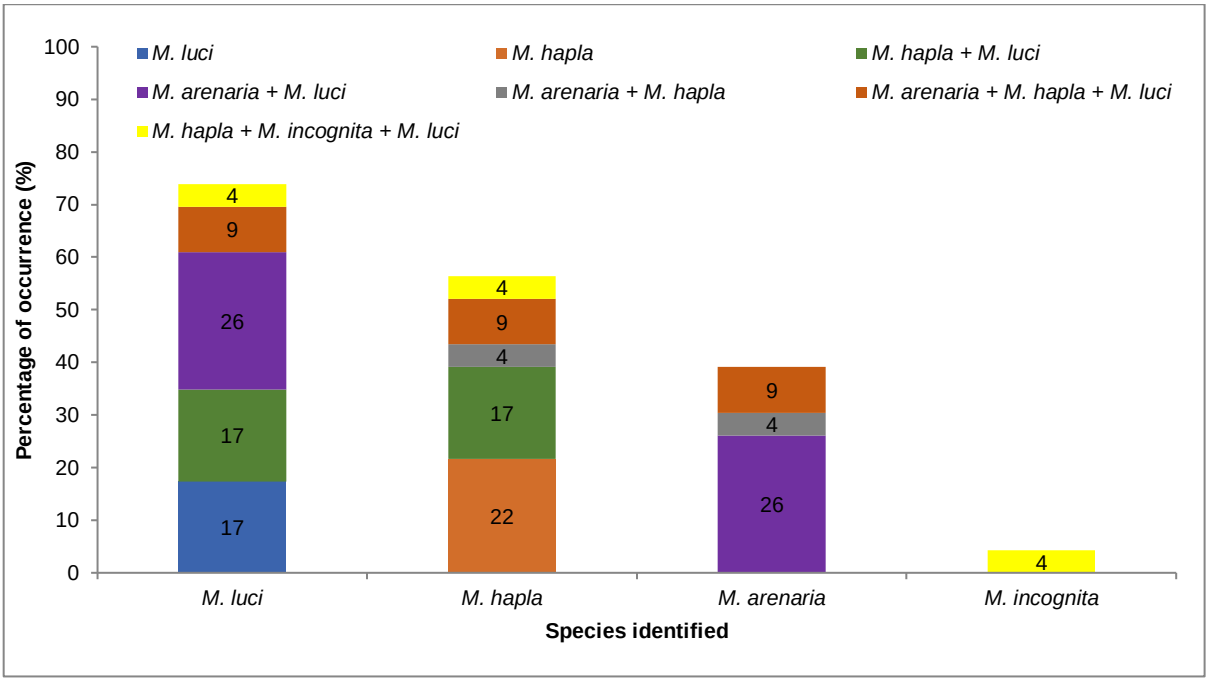

Figure 4. Frequency of *Meloidogyne* spp. identified in kiwifruit orchards in Samsun, Türkiye.

All *Meloidogyne* spp. identified in this study were detected in Çarşamba District (Figure 1). *Meloidogyne hapla* was present in all surveyed districts except Ondokuzmayıs, where *M. arenaria* and *M. luci* occurred. *Meloidogyne luci* was also found in Atakum and Terme Districts. *Meloidogyne arenaria* was detected in Atakum, Çarşamba and Ondokuzmayıs Districts whereas *M. incognita* was only found in Çarşamba District.

Fruit yields were obtained from two orchards (Orchard A and B) at the harvest period to estimate yield losses caused by RKN in kiwifruit. The mean population densities (mean ± SD) of *Meloidogyne* in vines infested in orchard A and B were determined as 526 ± 345 and 333 ± 233 J2/100 mL soil, respectively. According to the survey study, *M. luci* and *M. hapla* were found in orchard A, and *M. arenaria, M. hapla* and *M. luci* in orchard B (Table 1). In both orchards, the presence of *Meloidogyne* spp. was significantly reduced kiwifruit yield ($P < 0.05$) (Table 2). Relative yield losses compared to non-infested plants were determined as 36% in orchard A and 49% in orchard B. Fruits harvested from nematode-infested vines were smaller than that of non-infested vines in the orchards having the same cultural treatments (Figure 5).

Table 2. Kiwifruit yields (kg/vine, mean ± SD, n = 4) of *Meloidogyne*-infested and non-infested vines in two orchards in Samsun Province, Türkiye

Kiwifruit	Orchard A	Orchard B
Nematode-infested tree	100 ± 23	32 ± 9
Non-infested tree	158 ± 18	62 ± 10

Figure 5. Fruits harvested from *Meloidogyne*-infested (upper row) and non-infested (bottom row) vines in a kiwifruit orchard in Samsun, Türkiye.

Discussion

The present study revealed the wide distribution of RKN in kiwifruit plantations of Samsun Province. In addition to *M. arenaria, M. hapla* and *M. incognita* that have been which have previously been identified in kiwifruit orchards of Türkiye (Akyazı & Felek, 2013; Akyazi et al., 2017; Evlice & Özdemir, 2021), *M. luci* was reported for the first time with this study. Also, it was determined as the most common *Meloidogyne* spp. in kiwifruit orchards. Similar results on the prevalent of *M. luci* in Samsun were obtained in previous survey studies conducted on vegetable plants in greenhouses and open fields (Aydınlı & Mennan, 2016; Aydınlı, 2018). The esterase phenotype L3 is species-specific and the most useful character for differentiating *M. luci* from other species (Carneiro et al., 2014; Gerič Stare et al., 2017). An atypical (unidentified) population with L3 esterase phenotype on kiwifruit was detected first time by Somavilla et al. (2011) in Brazil. Subsequently, this population was reported as *M. luci* by Carneiro et al. (2014) in the original description of this nematode species. Before this description, a population of *Meloidogyne* obtained from soil sample of kiwifruit in Greece was reported as *M. ethiopica* by Conceição et al. (2012). However, this population was reclassified as *M. luci* along with all *M. ethiopica* populations detected in Europe (Gerič Stare et al., 2017). To our knowledge, the present study represents the third detection of *M. luci* on kiwifruit.

Taking into account the geographical distribution of RKN species reported on kiwifruit in the world, *M. hapla* is the most prevalent species and reported from Brazil (Somavilla et al., 2011), Chile (Philippi et al., 1996; Carneiro et al., 2007), France (Scotto La Massese, 1973), Greece (Tzortzakakis et al., 2011), India (Waliullah, 2005), Iran (Maafi & Mahdavian, 1997), Italy (Vovlas & Roca, 1976), New Zealand (Watson et al., 1992), South Africa (Shokoohi & Mashela, 2020), South Korea (Ma et al., 2007), Spain (Pinochet et al., 1990; Verdejo-Lucas, 1992) and Türkiye (Akyazi et al., 2017; Evlice & Özdemir, 2021). This nematode species had the widest distribution, occurring in five of the six surveyed districts, in this study, although it was the second highest frequency. With the third highest frequency, *M. arenaria* was detected in three districts of Samsun. Globally, *M. arenaria* has been recorded from kiwifruit in Australia (Castillo et al., 1993), Brazil (Somavilla et al., 2011), Iran (Maafi & Mahdavian, 1997), Italy (Castillo et al., 1993), Spain (Verdejo-Lucas, 1992), the USA (Haygood et al., 1990) and Türkiye (Akyazi et al., 2017; Evlice & Özdemir, 2021). In a survey conducted by Somavilla et al. (2011) in the Brazil, this nematode was detected as the most frequent *Meloidogyne* spp. in the samples. Akyazi et al. (2017) reported *M. arenaria* and *M. hapla* in kiwifruit roots for the first time in Türkiye, where both species were obtained from a commercial orchard in the neighboring province of Ordu. Evlice & Özdemir (2021) reported *Meloidogyne* infestation in four of eight sampled orchards in Bartın, Düzce, and Zonguldak from the Black Sea Region of Türkiye, and detected *M. hapla* in two orchards from Düzce and Zonguldak, where one of them also had *M. incognita*. The remaining infested orchards had *M. incognita*. In the first study conducted in kiwifruit orchards of Türkiye for RKN,

only *M. incognita* was found in all surveyed 17 orchards from Ordu (Akyazı & Felek, 2013). However, in the present study, *M. incognita* occurred only in one kiwifruit orchard from Çarşamba, where multiple RKN species infestation was found. The low frequency of this nematode in Samsun was also reported in recent surveys conducted in vegetable areas (Aydınlı & Mennan, 2016; Aydınlı, 2018). *Meloidogyne incognita* has also been found in Australia (Castillo et al., 1993), Brazil (Somavilla et al., 2011), Chile (Philippi et al., 1996), China (Tao et al., 2017), India (Khan, 2000), Iran (Maafi & Mahdavian, 1997), Italy (Castillo et al., 1993), Spain (Verdejo-Lucas, 1992) and the United States (Haygood et al., 1990).

Two or three nematode species were detected together in many orchards in this study. The occurrence of multiple *Meloidogyne* spp. in a kiwifruit orchard was also recorded in previous survey studies in kiwifruit orchards of Türkiye (Akyazi et al., 2017; Evlice & Özdemir, 2021). The multiple species that occurred in a kiwifruit orchard have also been reported in other countries (Verdejo-Lucas, 1992; Carneiro et al., 2007; Somavilla et al., 2011). The situation reveals that species identification must be performed on several specimens obtained from the same orchard since the accurate diagnosis of the species composition is required for developing appropriate nematode management practices (Kolombia et al., 2017).

Assessment of crop damage is a critical factor in deciding the phytosanitary importance of a pest species (Singh et al., 2013). In kiwifruit, there is little data on the damage potential of *Meloidogyne* (Di Vito et al., 1988; Philippi & Budge, 1992). In a pot experiment on the relationship between the initial population densities of *M. incognita* and plant growth in kiwifruit seedlings, Di Vito et al. (1988) found a growth suppression of 40 and 55% at eight and 32 eggs and juveniles per mL soil, respectively. However, Philippi & Budge (1992) indicated tolerance of kiwifruit seedlings to *M. hapla* in pot trials. Similarly, Pinochet et al. (1990) observed that the nematode population did not affect vegetative growth, yield, or fruit size, indicating the tolerance of plants. However, this consideration was not based on data. In our study, fruit yield decreased 36% in orchard A and 49% in orchard B in the *Meloidogyne*-infested vines compared to non-infested vines. To our knowledge, this is the first report on fruit yield losses caused by *Meloidogyne* in kiwifruit globally. However, yield loss was not associated with any RKN species since species identification was not done in sampled vines in harvest time.

The distribution of RKN in kiwifruit orchards in the Black Sea Region reveals a problem that should be addressed to ensure kiwifruit productivity in the near future. Currently, no registered nematicide is available for use on kiwifruit in Türkiye. Therefore, it is essential to be aware of the preventive measures. Especially, producers should obtain the seedlings free of nematode in planting in new areas. Only two kiwifruit orchards in this study, belonging to the same producer, were not infested with RKN. In this case, the producer had produced the seedlings himself whereas other producers obtained their seedlings from nurseries. The dissemination and wide distribution of RKN in a kiwifruit orchard and kiwifruit-producing areas of Samsun is most likely due to the planting of infested seedlings.

Acknowledgments

The authors thank Mr. İrfan Öztürk (Çarşamba Directorate of County Agriculture and Forestry, Samsun, Türkiye), Ms. Fadime Şen, Mr. Hoshang Kanabi Hamad, Mr. Hissein Mahamad Haroun and Ms. Merve Gümüş (Ondokuz Mayıs University, Samsun, Türkiye) for their assistance throughout sample collection.

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