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Original article (Orijinal araştırma)

Biochemical and molecular identification of root-knot nematodes in greenhouse vegetable areas of Eastern Mediterranean Region (Turkey)[1]

Doğu Akdeniz Bölgesi (Türkiye) örtüaltı sebze alanlarında kök-ur nematodlarının biyokimyasal ve moleküler tanımlanması

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Abstract

Root-knot nematode surveys were conducted during the growing seasons of 2017 and 2018 to cover the greenhouse vegetable areas in Mersin, Hatay and Adana Provinces in the Eastern Mediterranean Region (Turkey). A total of 46 root-knot nematode populations were characterized using biochemical and molecular diagnostic techniques. DNA extraction was done from second-stage juvenile and in molecular tests using SCAR primers, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne javanica* (Treub) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne hapla* Chitwood, 1949 and *Meloidogyne ethiopica* Whitehead, 1968 (Tylenchida: Meloidogynidae) species were screened. Samples obtained from thirty-nine regions were identified as *M. incognita* (85%) and seven as *M. javanica* (15%). In addition, young females obtained from all regions were biochemically analyzed using the polyacrylamide gel electrophoresis diagnostic method. The esterase enzyme profile was examined to identify the *M. incognita, M. javanica* and *M. ethiopica* groups. Esterase phenotype I1 band was observed in 62% of *M. incognita* populations and esterase phenotype I2 band was observed in 39%. Esterase phenotype J3 band was detected in all *M. javanica* populations. *M. javanica* and *M. incognita* were verified and also supported by molecular and biochemical methods in the Eastern Mediterranean Region.

Keywords: *Meloidogyne*, PAGE, PCR, root-knot nematodes, vegetable

Öz

Kök ur nematodu sürvey çalışmaları Doğu Akdeniz Bölgesi (Türkiye)'nde bulunan Mersin, Hatay ve Adana illerinde yoğun olarak sebze üretimi yapılan sera alanlarını kapsayacak şekilde 2017 ve 2018 yılı üretim sezonu boyunca yapılmıştır. Biyokimyasal ve moleküler tanılama teknikleri kullanılarak toplam 46 kök-ur nematodu popülasyonu karakterize edilmiştir. DNA ekstraksiyonu ikinci dönem larvalardan yapılmış olup, SCAR primerleri kullanılarak yapılan moleküler analizde *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne javanica* (Treub) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne hapla* Chitwood, 1949 ve *Meloidogyne ethiopica* Whitehead, 1968 (Nematoda: Heteroderidae) türleri taranmıştır. Sonuçlar incelendiğinde, otuz dokuz bölgeden elde edilen örnekler *M. incognita* (%85) olarak, yedi örnek ise *M. javanica* (%15) olarak tespit edilmiştir. Ek olarak, polyacrylamide jel elektroforez tanılama yöntemi kullanılarak tüm bölgelerden elde edilen genç dişi bireyler biyokimyasal olarak analiz edilmiştir. *Meloidogyne incognita, M. javanica* ve *M. ethiopica* grubu tanılamak için esteraz enzim profiline bakılmıştır. *Meloidogyne incognita* popülasyonlarında %62 oranında esteraz fenotip I1 bandı, %39 oranında ise esteraz fenotip I2 bandı gözlenmiştir. Bütün *M. javanica* popülasyonlarında esteraz fenotip J3 bandı tespit edilmiştir. *Meloidogyne javanica* ve *M. incognita*, Doğu Akdeniz Bölgesi'nde moleküler ve biyokimyasal yöntemlerle tanılanmış olup, bu iki yöntemden elde edilen sonuçlar birbirini desteklemektedir.

Anahtar sözcükler: *Meloidogyn*e, PAGE, PCR, kök-ur nematodları, sebze

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Introduction

The total greenhouse vegetable production area in Turkey is about 80 kHa. Adana, Hatay and Mersin Provinces in the Eastern Mediterranean Region have about 30 kHa of greenhouse vegetable area and are one of the most important centers of Turkey in this respect (TUIK, 2021).

More than half of the world's agricultural lands are infested with root-knot nematodes characterized by forming galls on the roots of host plants (Taylor, 1987; Trudgill & Blok, 2001). The size of the damage caused by root-knot nematode varies according to the type of host and climatic conditions (Netscher & Sikora, 1990; Karssen & Moens, 2006; Greco & Di Vito, 2009; Collange et al., 2011).

Root-knot nematodes inhibit plant growth by reducing nutrient and water uptake. They also increase plant susceptibility to fungal and bacterial disease due to the lesions they caused (Netscher & Sikora, 1990; Trudgill & Blok, 2001). The correct identification of the species is the most important for determining the economic control methods. There has been a considerable increase in the number of root-knot nematode species recognized through the development of diagnostic techniques in recent years. Hunt & Handoo (2009) and Jones et al. (2013) list more than 100 root-knot nematode species across the world.

With the development of diagnostic techniques, molecular and biochemical methods can make specific, reliable and accurate diagnoses in a short time. Markers such as SCAR, mtDNA, SSR and ISSR are currently used in molecular diagnosis of *Meloidogyne* spp. (Gözel et al., 2016). Also, root knot nematode ethiopica group [*Meloidogyne ethiopica* Whitehead, 1968, *Meloidogyne luci* Carneiro et al., 2014 (Nematoda: Heteroderidae)] which has been identified in recent years in Turkey and also some other European countries, can be distinguished only by its biochemical esterase enzyme profile (Aydınlı et al., 2013; Aydınlı & Mennan, 2016; Gerič Stare et al., 2017, 2018, 2019; Aydınlı, 2018; Gürkan et al., 2019). Esterase enzyme profiles are used as species-specific markers and support other identification methods (Butler et al*.,* 1981; Leslie et al., 1982; Barker, 1985; Esbenshade & Triantaphyllou, 1986; McGhee & Cottrell, 1986).

In studies conducted in Turkey, ten *Meloidogyne* spp., viz., *M. incognita, M. javanica, M. arenaria, M. hapla, M. chitwoodi, M. artiellia, M. acrita, M. luci, M. exiqua* and *M. thamesi*, have been recorded (Diker, 1959; Yüksel, 1966; Öztüzün, 1970; Ertürk & Özkut, 1973; Yüksel, 1974; Gürdemir & Ağdacı, 1975; Hekimoğlu, 1975; Pehlivan & Kaşkavalcı, 1993; Di Vito et al., 1994; Elekçioğlu & Uygun, 1994; Elekcioglu et al., 1994; Mennan & Ecevit, 1996; Kaşkavalcı & Öncüer, 1999; Söğüt & Elekçioğlu, 2000; Kepenekci et al., 2002; Devran et al., 2009; Devran & Söğüt, 2009; Özarslandan et al., 2009; Özarslandan & Elekçioğlu, 2010; İmren et al., 2014; Çetintaş & Çakmak, 2016; Gürkan, 2017; Uysal et al., 2017; Aydınlı, 2018; Gürkan et al., 2019).

The Eastern Mediterranean Region is one of the most important greenhouse vegetable production areas of Turkey (Elekcioğlu & Uygun, 1994; Elekcioglu et al., 1994). Söğüt & Elekcioğlu (2000) previously identified root-knot nematodes by morphological and North Carolina host test methods in this region. It was reported that *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood*,* 1949 races 2 and 4, and *Meloidogyne javanica* (Treub, 1885) Chitwood*,* 1949 race 1 were common species, and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and *Meloidogyne hapla* Chitwood, 1949 were rare species.

The fact that *M. luci* was found for the first time in Samsun and later found in Osmaniye and Gaziantep, provinces close to the Eastern Mediterranean Region where the study was conducted, revealed the necessity of revision by making detailed scanning in the region (Aydınlı, 2018; Gürkan et al., 2019). The aim of this study was to update root-knot nematode species and current species distributions in the Eastern Mediterranean region by using molecular scar primer and biochemical esterase enzyme.

Materials and Methods

Survey

One hundred root samples (20 from Adana, 75 from Mersin and 5 from Hatay) were collected in April to June 2018 in the areas where greenhouse of tomato, pepper, cucumber and eggplant vegetables were

grown in the Eastern Mediterranean Region (Bora & Karaca, 1970) (Figure 1). Root-knot nematode adult female, egg mass and second-stage juvenile were isolated from 46 samples (Table 3). The other 54 samples were not infected.

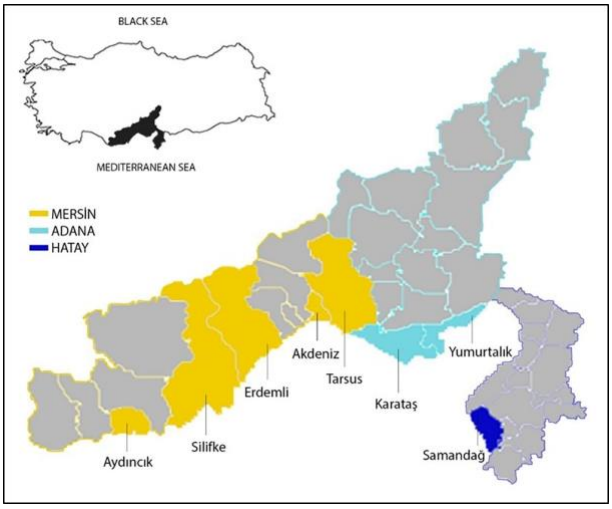

Figure 1. Districts surveyed in the greenhouse vegetable areas of the Eastern Mediterranean Region.

DNA isolation

DNA was extracted from second-stage juvenile of root-knot nematode populations according to the CTAB method stated by Ahrens & Seemüller (1992).

PCR studies

In the identification of root-knot nematodes, PCR was performed using METH F-METH R, MIF-MIR, FJAV-RJAV, FAR-RAR, and JMV1-JMV2-JMVhapla species-specific primers given in Table 1.

Table 1. Species-specific primers used in PCR studies to identify *Meloidogyne* spp.

Primers	Primer sequences (5'-3')	Fragments (bp)	*Meloidogyne* sp.	Reference
FJAV	GGTGCGCGATTGAACTGAGC	670	*M. javanica*	Zijlstra et al., 2000
RJAV	CAGGCCCTTCAGTGGAACTATAC			
FAR	TCGGCGATAGAGGTAAATGAC	420	*M. arenaria*	Zijlstra et al., 2000
RAR	TCGGCGATAGACACTACAAACT			
METH F	ATGCAGCCGCAGGGAACGTAGTTG	350	*M. ethiopica*	Correa et al., 2014
METH R	TGTTGTTTCATGTGCTTCGGCATC			
MIF	GTGAGGATTCACCTCCCCAG	999	*M. incognita*	Meng et al., 2004
MIR	ACGAGGAACATACTTCTCCGTCC			
JMV1	GGATGGCGTGCTTTCAAC	540, 670 and 440	*M. chitwoodi, M. fallax* and *M. hapla*	Wishart et al., 2002
JMV2	TTTCCCCTTATGATGTTTACCC			
JMVhapla	AAAAATCCCCTCGAAAAATCCACC			

In the PCR mix, 1 µl dNTPs (10 mM), 5 µl Dream Taq green buffer (10X), 2 µl DNA, 1 µl reverse primer (10 pmol), 0.25 µl Dream Taq DNA polymerase (5 u/µl), 1 µl forward primer (10 pmol) and 39.75 µl ddH_2O were added to make up the mixture to 50 µl. Different PCR cycles were used according to relevant literature for each primer pair (Table 2).

Table 2. Thermo-cycles applied for species-specific primer pairs

MIF-MIR & FJAV-RJAV	JMV1-JMV2-JMVhapla	FAR / RAR	METH F / METH R	
95°C 3 min	95°C 3 min	95°C 3 min	95°C 3 min	
95°C 60 s 59°C 60 s 72°C 60 s	95°C 45 s 53°C 45 s 72°C 45 s	95°C 30 s 56°C 30 s 72°C 45 s	95°C 45 s 60°C 45 s 72°C 45 s	} 35 cycles
72°C 7 min	72°C 7 min	72°C 7 min	72°C 7 min	

Agarose gel electrophoresis studies were conducted according to the method proposed by Galitelli & Minafra (1994). Agarose gel concentrations of 1% for *M. incognita*, 1.5% for *M. javanica* and 2% for *M. arenaria, M. hapla, M. chitwoodi* and *M. fallax* were adjusted according to the expected PCR product sizes by used primers and has been visualized in a UV transilluminator after stained with ethidium bromide.

Polyacrylamide gel electrophoresis

The study was performed on young females obtained from root samples with galls under a stereo binocular microscope. One young female was collected from each sample and Bio-Rad Mini-Protein II electrophoresis was run with 10 µl of extraction buffer according to the polyacrylamide gel electrophoresis (PAGE) method developed by Esbenshade & Triantaphyllou (1985). Five young females of *M. javanica* from pure culture were used as a control. The voltage was maintained at 80 v for the first 13 min and then increased to 200 v for the next 45 min. To visualize the esterase phenotype, gels was taken from electrophoresis and placed in the dying solution and kept in the dark for 30 min. After that, the gels were transferred to the solution containing 10% glycerol, 20% ethanol and 70% distilled H_2O fixative, and esterase phenotype bands were visualized in white light (Esbenshade & Triantaphyllou, 1985; Fargette 1987). The proportional mobilities of the bands were calculated and the esterase phenotype bands were designed according to Esbenshade & Triantaphyllou (1985) (Figure 6).

Results

In the sampling made from greenhouse vegetable production areas in the Eastern Mediterranean Region, the prevalence of root-knot nematodes was 46%. In the study, all samples were screened with the SCAR primer indicated in Table 2 and only *M. incognita* and *M. javanica* species were identified. Esterase enzyme profiles of 46 populations identified by PCR from root-knot nematode samples collected from the Eastern Mediterranean Region were monitored by the PAGE method. The identification key of the esterase profiles of the reference isolates used as a result of the tests was designed according to Esbenshade & Triantaphyllou (1985) (Figure 2).

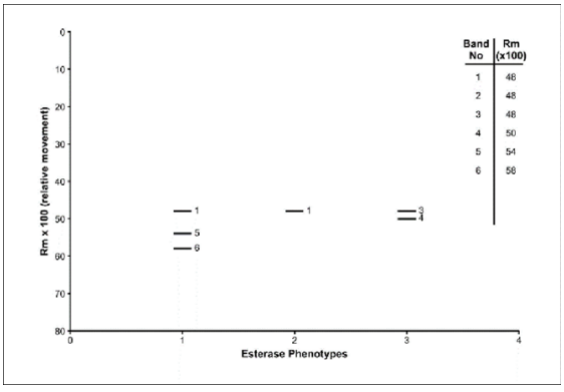

Figure 2. *Meloidogyne* spp. collected from vegetable greenhouses in the Eastern Mediterranean Region. The esterase enzyme phenotypes identification key was designed according to Esbenshade & Triantaphyllou (1985). Two esterase phenotypes of *M. incognita*, I1(1) and I2 (3 and 4); and *M. javanica*, J3 (1, 5 and 6).

Meloidogyne incognita

Meloidogyne incognita was identified in 39 samples collected from Karataş, Tarsus, Adanalıoğlu, Kazanlı, Erdemli, Aydıncık and Samandağ, and the prevalence was 85% (Table 3). For the *M. incognita* population, after the PCR study using the MIF-MIR primer pair developed by Meng et al. (2004), a DNA band with a length of 999 bp was visualized (Figure 3). It was supported by the PAGE method that 39 *M. incognita* isolates, which were identified as a result of molecular studies, also had I1 or I2 esterase profiles and that these isolates *were M. incognita* (Figure 4). It was found that I1 esterase enzyme phenotype was 62% and I2 esterase enzyme phenotype was 39%.

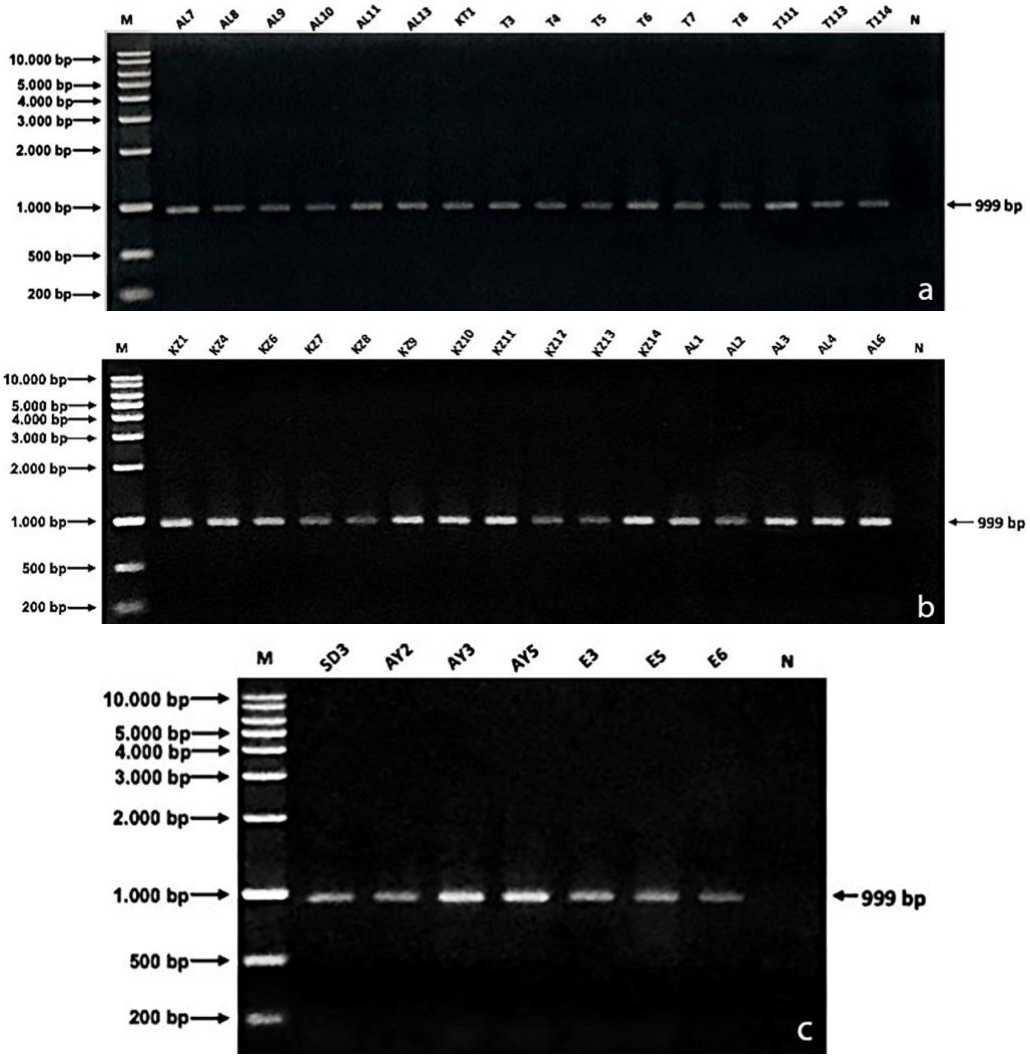

Figure 3. PCR products (999 bp) of *Meloidogyne incognita* species-specific MIR-MIF primers in AL7-E6 samples. (M, 1000 bp DNA marker; and N, dd-water).

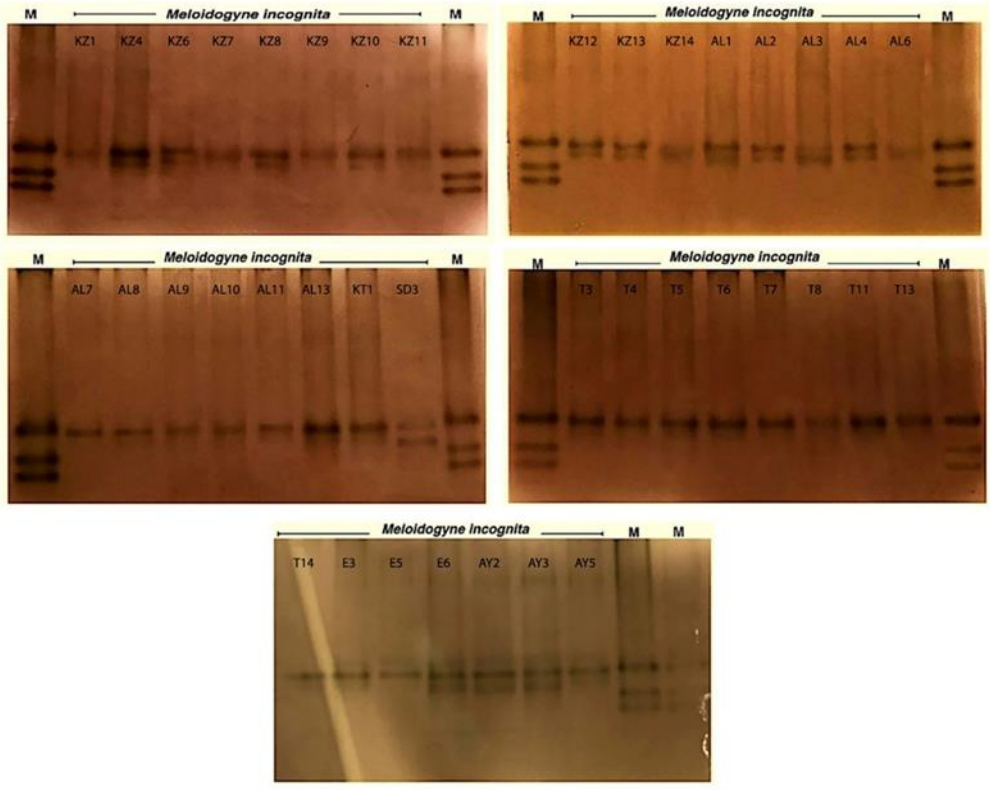

Figure 4. *Meloidogyne incognita* 24 population I1 esterase enzyme phenotypes: KZ1, KZ7, KZ9, KZ11, KZ14, AL6, AL7, AL8, AL9, AL10, AL11, AL13, KT1, T3, T4, T5, T6, T7, T8, T11, T13, T14, E3, E5. 15 population I2 esterase enzyme phenotypes: KZ4, KZ6, KZ8, KZ10, KZ12, KZ13, AL1, AL2, AL3, AL4, SD3, E6, AY2, AY3, AY5. M, marker-reference population (*Meloidogyne javanica*).

Meloidogyne javanica

Meloidogyne javanica was diagnosed in 7 of 46 samples collected from Kazanlı, Aydıncık, Erdemli and Samandağ and the prevalence was 15%. To identify *M. javanica*, after the PCR study using the FJAV/RJAV primer pair developed by Zijlstra et al. (2000), the expected DNA band of 670 bp was displayed as a result of the PCR study (Figure 5).

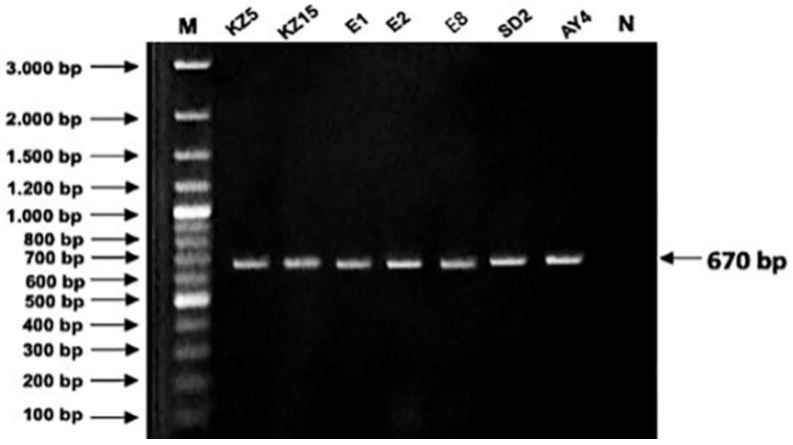

Figure 5. PCR products of KZ5-AY4 samples at 670 bp with the *Meloidogyne javanica* FJAV-RJAV primers. M, 100 bp DNA ladder; and N, dd-water.

This result was supported by the PAGE method that 7 *M. javanica* isolates, which were identified as a result of molecular studies, had J3 esterase profile and that these isolates were *M. javanica* as a result (Figure 6).

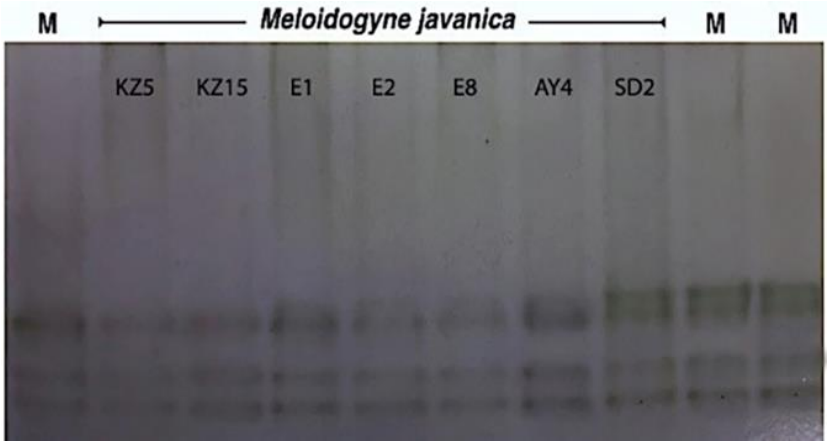

Figure 6. J3 phenotype of esterase enzyme profile of *Meloidogyne javanica* isolates in polyacrylamide gel. M, marker-reference population (*Meloidogyne javanica*).

The results obtained from molecular and biochemical analyzes are presented in Table 3.

Table 3. Comparison of the molecular and biochemical analysis results (as positives and negatives) for *Meloidogyne* spp. of the isolates collected from the Eastern Mediterranean Region

Location	Code	Host	Esterase			Primers		*Meloidogyne* sp.	Coordinates	
			I1	I2	J3	MIF-MIR	FJAV-RJAV		N	E
Adana, Karataş	KT1	Tomato	+	-	-	+	-	*M. incognita*	36°71'18"	35°14'51"
	KZ1	Pepper	+	-	-	+	-	*M. incognita*	36°82'57"	34°76'68"
	KZ4	Pepper	-	+	-	+	-	*M. incognita*	36°82'78"	34°73'37"
	KZ5	Pepper	-	-	+	-	+	*M. javanica*	36°82'23"	34°77'56"
	KZ6	Pepper	-	+	-	+	-	*M. incognita*	36°82'15"	34°77'41"
	KZ7	Pepper	+	-	-	+	-	*M. incognita*	36°82'23"	34°76'50"
	KZ8	Pepper	-	+	-	+	-	*M. incognita*	36°83'26"	34°74'19"
Mersin, Kazanlı	KZ9	Pepper	+	-	-	+	-	*M. incognita*	36°81'39"	34°75'42"
	KZ10	Pepper	-	+	-	+	-	*M. incognita*	36°83'85"	34°74'18"
	KZ11	Pepper	+	-	-	+	-	*M. incognita*	36°81'37"	34°78'24"
	KZ12	Pepper	-	+	-	+	-	*M. incognita*	36°81'37"	34°78'24"
	KZ13	Pepper	-	+	-	+	-	*M. incognita*	36°81'04"	34°78'90"
	KZ14	Pepper	+	-	-	+	-	*M. incognita*	36°82'48"	34°76'73"
	KZ15	Pepper	-	-	+	-	+	*M. javanica*	36°82'23"	34°77'56"

Table 3. Continued

Location	Code	Host	Esterase			Primers		*Meloidogyne* sp.	Coordinates	
			I1	I2	J3	MIF-MIR	FJAV-RJAV		N	E
Mersin, Adanalıoğlu	AL1	Pepper	-	+	-	+	-	*M. incognita*	36°83'63"	34°80'90"
	AL2	Pepper	-	+	-	+	-	*M. incognita*	36°83'63"	34°81'00"
	AL3	Pepper	-	+	-	+	-	*M. incognita*	36°83'56"	34°81'09"
	AL4	Pepper	-	+	-	+	-	*M. incognita*	36°83'69"	34°81'29"
	AL6	Pepper	+	-	-	+	-	*M. incognita*	36°83'40"	34°81'74"
	AL7	Pepper	+	-	-	+	-	*M. incognita*	36°83'15"	34°81'50"
	AL8	Pepper	+	-	-	+	-	*M. incognita*	36°83'13"	34°81'28"
	AL9	Pepper	+	-	-	+	-	*M. incognita*	36°83'07"	34°81'62"
	AL10	Pepper	+	-	-	+	-	*M. incognita*	36°82'79"	34°81'92"
	AL11	Pepper	+	-	-	+	-	*M. incognita*	36°82'73"	34°82'58"
	AL13	Pepper	+	-	-	+	-	*M. incognita*	36°81'26"	34°81'55"
Mersin, Tarsus	T3	Eggplant	+	-	-	+	-	*M. incognita*	36°79'85"	34°87'90"
	T4	Pepper	+	-	-	+	-	*M. incognita*	36°79'77"	34°87'87"
	T5	Eggplant	+	-	-	+	-	*M. incognita*	36°79'77"	34°87'81"
	T6	Eggplant	+	-	-	+	-	*M. incognita*	36°79'67"	34°87'81"
	T7	Eggplant	+	-	-	+	-	*M. incognita*	36°79'63"	34°87'83"
	T8	Pepper	+	-	-	+	-	*M. incognita*	36°79'70"	34°87'87"
	T11	Pepper	+	-	-	+	-	*M. incognita*	36°80'27"	34°86'63"
	T13	Pepper	+	-	-	+	-	*M. incognita*	36°80'26"	34°86'67"
	T14	Pepper	+	-	-	+	-	*M. incognita*	36°80'30"	34°86'64"
Mersin, Erdemli	E1	Tomato	-	-	+	-	+	*M. javanica*	36°60'46"	34°26'14"
	E2	Tomato	-	-	+	-	+	*M. javanica*	36°60'55"	34°26'22"
	E3	Tomato	+	-	-	+	-	*M. incognita*	36°60'16"	34°27'37"
	E5	Tomato	+	-	-	+	-	*M. incognita*	36°60'26"	34°26'90"
	E6	Tomato	-	+	-	+	-	*M. incognita*	36°60'58"	34°26'42"
	E8	Tomato	-	-	-	-	+	*M. javanica*	36°60'54"	34°25'26"
Mersin, Aydıncık	AY2	Cucumber	-	+	-	+	-	*M. incognita*	36°16'13"	33°33'84"
	AY3	Cucumber	-	+	-	+	-	*M. incognita*	36°16'08"	33°36'31"
	AY4	Cucumber	-	-	+	-	+	*M. javanica*	36°16'25"	33°38'28"
	AY5	Eggplant	-	+	-	+	-	*M. incognita*	36°15'90"	33°37'55"
Hatay, Samandağ	SD2	Tomato	-	-	+	-	+	*M. javanica*	36°07'30"	35°98'57"
	SD3	Tomato	-	+	-	+	-	*M. incognita*	36°06'92"	35°98'80"

Discussion

Overall, 46 of 100 root samples collected from vegetable growing greenhouses in the Eastern Mediterranean Region were found to be heavily infested with root-knot nematodes. *Meloidogyne incognita*

was determined as the dominant species in the region. *Meloidogyne incognita* was identified as the common species in Adanalıoğlu, Kazanlı and Tarsus districts in Mersin, where pepper cultivation is intense. Also, *M. incognita* was detected in Aydıncık, Erdemli (Mersin), Karataş (Adana) and Samandağ (Hatay) regions where cucumber, tomato and eggplant cultivation are intense. In the Eastern Mediterranean Region, the prevalence of *M. incognita* was 42% in the Söğüt & Elekçioğlu (2000) and as 62% in the Özarslandan & Elekcioğlu (2010). In this study, *M. incognita* found as the dominant species with a very high rate of 85%. Similarly, Gürkan et al. (2019) determined that the prevalence of *M. incognita* was 93% in Gaziantep and 90% in Osmaniye.

The rate of *M. incognita* infestation in greenhouse vegetable cultivation in the Western Mediterranean Region was previously reported as 64% (Devran & Söğüt, 2009) and 37% in the Lakes Region (Uysal et al., 2017). Kaşkavalcı & Öncüer (1999) report the prevalence of *M. incognita* in the Aegean Region as 80% whereas Aydınlı & Mennan (2016) in the Central Black Sea vegetable areas as 4%. It is thought that the reason for the low prevalence in the latter case may be the identification of the species previously identified as *M. incognita or M. ethiopica as M. ethiopica* group (*M. ethiopica, M. luci* and *Meloidogyne inornata* Lordello, 1956).

Meloidogyne javanica was identified in only seven samples collected from Kazanlı, Aydıncık, Erdemli (Mersin) and Samandağ (Hatay). The prevalence of *M. javanica* in the Eastern Mediterranean Region was 15%. In the same region, Söğüt & Elekçioğlu (2000) found the prevalence of *M. javanica* to be 55% and Özarslandan & Elekcioğlu (2010) as 39%. Söğüt & Elekçioğlu (2000), Devran & Söğüt (2011), Uysal et al. (2017), Gürkan et al. (2019) and Dinçer (2021) determined that *M. javanica* race 1 was dominant, but different races of *M. javanica* were reported both in the region and in different regions in the same studies. In this study, a race test was not conducted, but *M. javanica* was detected in the samples taken from the pepper fields of the Kazanlı district of Mersin.

In the present study, the prevalence of *M. javanica* in the Eastern Mediterranean Region was found to have decreased considerably. Similarly, Gürkan et al. (2019) found low rates of *M. javanica* infestation in Gaziantep and Osmaniye were 7% and 10%, respectively. Devran & Söğüt (2009) reported the prevalence of *M. javanica* on the coastline of the Western Mediterranean Region was 28% and Uysal et al. (2017) reported it as 37% in the Lakes Region. The prevalence of *M. javanica* in the Central Black Sea vegetable areas was 15% and in the Aegean Region it was 12% (Aydınlı & Mennan, 2016). In the present study, 62% I1 esterase bands in 24 populations and 39% I2 esterase bands in 15 populations were detected in the esterase enzyme phenotypes of 39 *M. incognita* samples identified by PAGE. In other PAGE studies conducted in Turkey, it was reported that *M. incognita* I2 esterase enzyme phenotype is more common (Aydınlı & Mennan, 2016; Cetintas & Cakmak, 2016; Gürkan et al., 2019). Esterase enzyme phenotypes were reported by Pais & Abrantes (1989), Carneiro et al. (1996, 2000), Castro et al. (2003), Cofcewicz et al. (2005) and Cetintas & Cakmak (2016). The two esterase enzyme phenotypes found in 2017 were similar to I1, I2 and J3 and were consistent those studies.

Conclusion

In recent years, *M. luci* have been identified in studies conducted with PAGE, esterase phenotype differences in the Central Black Sea and Eastern Mediterranean Regions and in Osmaniye and Gaziantep Provinces (Aydınlı & Mennan, 2016; Gürkan et al., 2019). Both nematodes show high similarity at the morphological and genetic level, and it was been reported that the best way to distinguish them is the esterase enzyme phenotype (Gerič Stare et al., 2018, 2019). Similarly, *M. ethiopica* has been redefined as *M. luci* in Slovenia, Greece and Italy. It has also been reported that *M. ethiopica* populations in Turkey, reclassified as *M. luci* (Gerič Stare et al., 2017).

Root-knot nematodes cause great economic yield losses, especially in vegetable growing areas. In addition, because they are soil-borne and their hosts range is wide, it is difficult and costly to control in and eradicate from soil. Accurate identification is of great importance, especially in resistance cultivar studies and commercial use of developed cultivars. The use of plant species resistant to root-knot nematode in the region is important in terms of determining control strategies of this pest.

In the present study, *M luci* was not found in the region using PAGE method. Rather, the previously identified species, *M incognita*, was found to be a highly dominant species. This research showed that invasive pests with complex host-pathogen relationships, such as root-knot nematodes, need to be regularly surveyed with the best available diagnostic methods.

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