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*Original article (Orijinal araştırma)*

# *Meloidogyne* species infesting tomatoes, cucumbers and eggplants grown in Kahramanmaraş Province, Turkey[1]

## Kahramanmaraş bölgesinde tarımı yapılan domates, hıyar ve patlıcan bitkilerinde mevcut *Meloidogyne* türleri

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## Summary

In 2009 and 2010, 107 samples of roots and associated soil were collected from vegetable-growing fields in Andırın, Narlı, Pazarcık and Türkoğlu of Kahramanmaraş Province, Turkey. Populations of root-knot nematode were found in tomatoes, eggplants and cucumbers and the species identified using isozymes analysis, mainly esterase resolved by PAGE (Polyacrylamide Gel Electrophoresis) and perineal patterns of nematode females. A single species of root-knot nematode, *Meloidogyne incognita* was identified from the samples collected. Two esterase phenotypes, one with a single band (I1) and the other with double bands (I2) were found at proportions of 8.5 and 15.8% of total samples, respectively. About 24% of the fields were found to be infested.

**Keywords:** Esterase phenotypes, identification, *Meloidogyne incognita*, perineal patterns

## Özet

Kahramanmaraş'ın Andırın, Narlı, Pazarcık ve Türkoğlu bölgelerinden 2009-2010 yıllarında sebze alanlarından toplam 107 topraklı bitki kök örneği toplanmıştır. Domates, patlıcan ve hıyar bitkilerinden elde edilen nematodlar PAGE (Poliakrilamid Jel Elektroforez) belirlenen esteraz enzim fenotipleri ve perineal (anal) kesit yöntemi ile tür teşhisleri yapılmıştır. Örneklerde mevcut olan nematodların *Meloidogyne incognita* tek türüne ait olduğu bulunmuştur. Bu nematoda ait tek ve çift bantlı olmak üzere iki ayrı esteraz fenotipi olan I1 ve I2 belirlenmiş olup, bunların tüm örnekler içinde sırası ile %8.5 ve 15.8 oranında olduğu görülmüştür. Örneklenen tüm Kahramanmaraş bölgesindeki sebze alanlarının yaklaşık % 24'ünün ilgili kök-ur nematodu ile bulaşık olduğu tespit edilmiştir.

**Anahtar sözcükler:** Esteraz fenotipleri, teşhis, *Meloidogyne incognita*, anal kesit

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## Introduction

*Meloidogyne* is one of the most widespread and economically important plant- parasitic nematode genera and damages a wide variety of plants across the world (Sasser & Carter, 1985). Plant parasitic nematodes can cause a loss of 78 (Barker et al., 1994) to 100 billion USD (Sasser, 1987) annually. *Meloidogyne* spp. are believed to be a major contributor of this loss. More than 90 root-knot nematode species (*Meloidogyne* spp.) are reported across the world (Hunt & Handoo, 2009). The survey conducted in the International *Meloidogyne* Project in 75 countries found that *Meloidogyne incognita* Kofoid & White, 1919, *Meloidogyne javanica* Treub, 1885, *Meloidogyne arenaria* Neal, 1889 and *Meloidogyne hapla* Chitwood, 1949 were among the most common and economically important species of root-knot nematode in agricultural soils (Netscher & Sikora, 1990). Root-knot nematodes can be found in different regions of the world; however, crop losses caused by these nematodes are greatest in tropical regions (Johnson & Fassuliotis, 1984; Mai, 1985). *Meloidogyne incognita* and *M. javanica* are commonly found in tropical regions, whereas *M. hapla* is better adapted to temperate areas (Taylor & Sasser, 1978). *Meloidogyne incognita* has the widest geographic distribution of all the species described, followed closely by *M. javanica* and *M. arenaria* (Nestscher & Sikora, 1990). *Meloidogyne incognita* along with *Meloidogyne javanica* has been found infesting about 3000 plant species around the world.

Until 2014, eight species of root-knot nematodes (*Meloidogyne arenaria*, *M. artiellia*, *M. chitwoodi*, *M. ethiopica*, *M. exigua*, *M. hapla*, *M. incognita*, and *M. javanica*) have been detected in various agricultural areas of Turkey (Yüksel, 1974; Di Vito et al., 1994; Elekçioğlu & N. Uygun, 1994; Kaşkavalcı & Öncüer, 1999; Söğüt & Elekçioğlu, 2000; Devran et al., 2009; Devran & Söğüt, 2009; Özarslandan et al., 2009; Özarslandan & Elekçioğlu, 2010; Akyazı & Ecevit, 2011; Aydınlı et al., 2013; Kepenekçi et al., 2014). However, only five root-knot nematode species; *M. arenaria*, *M. hapla, M. incognita*, *M. javanica* and *M. thamesi* and have been reported infesting vegetables. Nonetheless, *M. arenaria* and *M. hapla* were found to be widespread but relatively rare (Yüksel, 1974; Elekçioğlu & Uygun, 1994).

Deciding on an effective and successful nematode control method depends directly on accurate problem recognition and knowledge of nematode species involved. Accurate identification of *Meloidogyne* spp. is very critical and important for designing and implementing control strategies such as integrated pest management, crop rotation, use of resistant cultivars, and plant breeding and regulatory programs (Roberts, 1992; Young, 1992; Sijmons et al., 1994; Gheysen et al., 1996; Tytgat et al., 2000; Coyne et al., 2009).

Identification of root-knot nematode species has mostly been performed by four procedures; isozymes, morphology of selected characters, molecular methods and host differential tests. The first two of these methods were applied in the current study. Isozyme phenotypes of nematode females, although long established, are still a useful method in nematology (Esbenshade & Triantaphyllou, 1985). Esterase (EST) and malate dehydrogenase (MDH) isozyme phenotypes (Dickson et al., 1970; Esbenshade & Triantaphyllou, 1985) resolved in polyacrylamide gel electrophoresis were found very effective, and fast in the identification and differentiation of root-knot nematodes species. These enzymes have been used successfully to identify different root-knot nematode species by many scientists from different countries across to the world (Fargette, 1987; Pais & Abrantes, 1989; Carneiro & Almeida, 2001; Castro et al., 2003; Cetintas et al., 2003, Cofcewicz et al., 2004, 2005; Brito et al., 2008). Furthermore, mitochondrial haplotypes used to identify some tropical species of *Meloidogyne*, was in total agreement with esterase analysis (Janssen et al., 2016).

Identification of *Meloidogyne* spp. by morphology, to some degree, is time-consuming and difficult (Taylor & Sasser, 1978; Jepson, 1987). Nevertheless, perineal patterns of females are one of the most frequently used tools for the morphological identification of root-knot nematodes, particularly for differentiating the four commonly found species, *M. arenaria*, *M. hapla, M. javanica* and *M. incognita* (Jepson, 1987; Hunt & Handoo, 2009). However, the method can sometimes be inconclusive for closely related *Meloidogyne* spp., since individuals within the same population often vary considerably (Zijlstra et al., 2000).

The objectives of this study were to determine the prevalence and geographical distribution of *Meloidogyne* spp. in Kahramanmaraş Province, South Mediterranean Region, Turkey, and to identify and characterize root-knot nematode species found infesting three vegetable crops (tomato, eggplant and cucumber) in this area.

## Materials and Methods

One hundred and seven samples of roots and associated soil were collected from tomato, eggplant and cucumber fields in the districts of Andırın, Narlı, Pazarcık and Türkoğlu of Kahramanmaraş Province, Turkey, in 2009 and 2010 (Table 1). To enable detection of gall formed by root-knot nematodes, the survey was conducted when plants were at least three months old after planting. This sampling period provided samples with the greatest amount of visible root-knot nematode galling, thus aiding with the recognition of root-knot nematode infested plants. Among five tomato cultivars grown in the region, Joker F1 and Servet F1 is intermediate resistance, and remaining cultivars, Bulanık, Pembe and Tokat F1 are susceptible to *M. incognita*. Additionally, two cucumber cultivars; Toros F1 and Başak F1, and six eggplant cultivars; Karnaz F1, Adana F1, Adana Dolmalık, Pala 49, Kemer and Anamur Karası are also susceptible to *M. incognita*. Consequently, most of host cultivars grown in the region are susceptible to the root-knot nematode. Also, given that most of the fields sampled were not routinely treated nematicides as this is not a common practice in the province, the samples provide a reliable indicator of root-knot nematode incidence in the area sampled.

Table 1. Sampling locations and detection of Meloidogyne in tomato, cucumber and eggplant crops in Kahramanmaraş Province, Turkey

| District | Location | Host plant | Samples collected | Infested samples | Incidence (%) and phenotype |
|---|---|---|---|---|---|
| Andırın | Merkez | Tomato | 4 | 0 | 0 |
| Andırın | Merkez | Cucumber | 2 | 0 | 0 |
| Andırın | Merkez | Eggplant | 3 | 0 | 0 |
| Kahramanmaraş | Akçakoyun Köyü | Tomato | 1 | 0 | 0 |
| Kahramanmaraş | Çiğli Köyü | Tomato | 5 | 5 | 100, I2 |
| Kahramanmaraş | Hasancıklı Köyü | Tomato | 2 | 0 | 0 |
| Kahramanmaraş | Hasancıklı Köyü | Cucumber | 1 | 0 | 0 |
| Kahramanmaraş | Hasancıklı Köyü | Eggplant | 2 | 0 | 0 |
| Kahramanmaraş | Kılılı Beldesi | Tomato | 2 | 0 | 0 |
| Narlı | Karabıyık Köyü | Tomato | 1 | 0 | 0 |
| Narlı | Narlı Yol Ayrımı | Tomato | 2 | 0 | 0 |
| Narlı | Narlı Yol Ayrımı | Cucumber | 1 | 0 | 0 |
| Narlı | Narlı Yol Ayrımı | Eggplant | 1 | 0 | 0 |
| Pazarcık | Salmanıpak Köyü | Tomato | 3 | 0 | 0 |
| Pazarcık | Salmanıpak Köyü | Cucumber | 3 | 0 | 0 |
| Pazarcık | Salmanıpak Köyü | Eggplant | 4 | 0 | 0 |
| Pazarcık | Kabarobası Köyü | Tomato | 4 | 0 | 0 |
| Pazarcık | Kabarobası Köyü | Cucumber | 4 | 0 | 0 |
| Pazarcık | Kabarobası Köyü | Eggplant | 4 | 0 | 0 |
| Pazarcık | Ulubahçe Köyü | Tomato | 1 | 0 | 0 |
| Pazarcık | Ulubahçe Köyü | Cucumber | 1 | 0 | 0 |
| Pazarcık | Ufacıklı Köyü | Cucumber | 1 | 0 | 0 |

Table 1. (Continued)

| District | Location | Host plant | Samples collected | Infested samples | Incidence (%) and phenotype |
|---|---|---|---|---|---|
| Pazarcık | Kurtdere Köyü | Tomato | 4 | 1 | 25, I2 |
| Pazarcık | Kurtdere Köyü | Cucumber | 3 | 0 | 0 |
| Pazarcık | Kurtdere Köyü | Eggplant | 3 | 0 | 0 |
| Türkoğlu | Aydın Kavak Köyü | Tomato | 8 | 8 | 100, I2 |
| Türkoğlu | Aydın Kavak Köyü | Cucumber | 3 | 3 | 100, I2 |
| Türkoğlu | Aydın Kavak Köyü | Eggplant | 9 | 9 | 100, I1 |
| Türkoğlu | Balık Alanı Köyü | Tomato | 4 | 0 | 0 |
| Türkoğlu | Balık Alanı Köyü | Cucumber | 3 | 0 | 0 |
| Türkoğlu | Beyoğlu Kasabası | Tomato | 7 | 0 | 0 |
| Türkoğlu | Beyoğlu Kasabası | Cucumber | 3 | 0 | 0 |
| Türkoğlu | Beyoğlu Kasabası | Eggplant | 5 | 0 | 0 |
| Türkoğlu | Çakallı Köyü | Tomato | 1 | 0 | 0 |
| Türkoğlu | Merkez | Tomato | 1 | 0 | 0 |
| Türkoğlu | Çoban Tepe Köyü | Tomato | 1 | 0 | 0 |

Plant roots with symptoms of root-knot nematode from each crop in each location was individually collected in polyethylene bags and kept in a cooler for further evaluation. The roots were washed gently in tap and individual root-knot nematode females were collected under light microscope with 40X magnification. Root samples with low infestation or with females not suitable for analysis, were cut into approximately 2 cm lengths, mixed and transferred to 16 cm diameter clay pots containing field soil to increase the population of nematodes. Root-knot nematode susceptible tomato (*Solanum lycopersicum* Mill. cv. SC 2121; Pinaper Tohumculuk, Adana, Turkey) seedlings were transplanted into the pots and maintained in a greenhouse. The pots were watered daily and fertilized as needed. Sixty days after transplanting, infested roots containing adult females were washed and nematode females recovered for study.

**Sample preparation, loading and electrophoresis**

Eight females were extracted from the roots of each plant for esterase study. The females were individually preserved (one female per tube) in 10 µl of extraction buffer (56% deionized water, 12% 0.5 M Tris-HCl, pH 6.8, 30% glycerol, 2% of 0.5% [w/v] bromophenol blue; BioRad, Hercules, CA, USA) in conical 50-µl microfuge tubes and frozen at -5°C. PAGE (polyacrylamide gel electrophoresis) were performed on a total of 512 young egg-laying females using a Bio-Rad mini-PROTEIN II (Bio-Rad) electrophoresis unit with 10 wells per gel. Before electrophoresis, the females were thawed and individually homogenized in a microhaematocrite plastic tube in 10 µl of extraction buffer, and then each was loaded into a well of a polyacrylamide gel consisting of a 4% stacking (pH 6.8) and a 8% separating (pH 8.8) sections in Tris-glycine buffer. Two females from a greenhouse isolate of *M. javanica* were individually extracted and stored in extraction buffer. These were used as standards in each gel. The standard *M. javanica* female extract was placed into wells 1 and 10. The voltage was maintained at 80 v for the first 15 min and increased to 200 v for the remainder of the run. Following electrophoresis, the gels were removed and placed in a staining solution to determine esterase activity (Harris & Hopkinson, 1976; Esbenshade & Triantaphyllou, 1985). Gels were stained in dark for about 30 min, and transferred to a fixative solution consisted of 10% glycerol, 20% ethyl alcohol and 70% distilled $H_2O$. Esterase phenotype bands were observed and gel photographs were taken under UV or white light (Figure 2). In addition, relative mobility of band(s) was calculated (Esbenshade & Triantaphyllou, 1985; Fargette 1987) and phenotype designations were assigned according to Esbenshade &Triantaphyllou (1985) (Figure 1).

### Perineal patterns

Females dissected from plant roots were selected randomly and fixed in TAF (7 ml 40% formaldehyde, 2 ml triethanolamine, 91 ml distilled water) solution until used. Overall, 387 of 567 females dissected from tomato, cucumber and eggplant were processed for examination of perineal patterns (Table 2). Females were cut in 45% lactic acid and mounted in glycerin (Taylor & Netscher, 1974; Hartman & Sasser, 1985). Perineal patterns examined under a light microscope as described by Eisenback et al. (1980, 1981) and Jepson (1987). Examination and photography of perineal patterns were completed within 12 h following slide preparation (Figure 3).

Table 2. The number of females and perineal patterns examined from tomato, cucumbers and eggplants sampled in Kahramanmaraş Province, Turkey

| Sample ID | Plant Source | Number of females collected | Number of perineal patterns examined |
|---|---|---|---|
| 09-TAKK1 | Tomato | 19 | 14 |
| 09-TAKK2 | Tomato | 30 | 26 |
| 09-TAKK3 | Tomato | 18 | 10 |
| 10-TAKK4 | Tomato | 20 | 16 |
| 10-TAKK5 | Tomato | 23 | 18 |
| 10-TAKK6 | Tomato | 21 | 13 |
| 10-TAKK7 | Tomato | 20 | 15 |
| 10-TAKK8 | Tomato | 25 | 17 |
| 09-TCI1 | Tomato | 17 | 13 |
| 09-TCI2 | Tomato | 11 | 9 |
| 09-TCI3 | Tomato | 19 | 15 |
| 10-TCI4 | Tomato | 13 | 8 |
| 10-TCI5 | Tomato | 15 | 14 |
| 10-TKU1 | Tomato | 5 | 3 |
| 09-CAKK1 | Cucumber | 13 | 9 |
| 10-CAKK2 | Cucumber | 10 | 6 |
| 10-CAKK3 | Cucumber | 8 | 5 |
| 09-EAKK1 | Eggplant | 25 | 18 |
| 09-EAKK2 | Eggplant | 19 | 10 |
| 09-EAKK3 | Eggplant | 30 | 16 |
| 09-EAKK4 | Eggplant | 29 | 13 |
| 10-EAKK5 | Eggplant | 32 | 19 |
| 10-EAKK6 | Eggplant | 17 | 14 |
| 10-EAKK7 | Eggplant | 26 | 17 |
| 10-EAKK8 | Eggplant | 38 | 21 |
| 10-EAKK9 | Eggplant | 35 | 23 |
| 10-EAKK10 | Eggplant | 29 | 25 |

## Results and Discussion

The survey revealed that about 24% of the fields sampled were infested with *Meloidogyne* populations. All populations were identified as *M. incognita*. Infected tomato, eggplant and cucumber fields were found in Aydın Kavak in Türkoğlu District of Kahramanmaraş Province. In Çiğli and Kurtepe villages in Pazarcık District of Kahramanmaraş Province infected tomato fields were found (Table 1). In Turkey, the use of esterase phenotypes for the identification of *Meloidogyne* species has been reported previously (Mennan et al., 2011; Aydınlı & Mennan, 2011, 2016; Çetintaş & Çakmak, 2011). The current study is the broadest study to determine the root-knot nematode incidence and to identify *Meloidogyne* species occurring in Kahramanmaraş Province by these two methods.

Two esterase phenotypes (Est = I1 and Est = I2) were detected among the populations of *M. incognita* (Figures 1 and 2). Relative migrations of the bands for phenotypes I1 and I2 were 49, and 49 and 51, respectively (Figure 1).

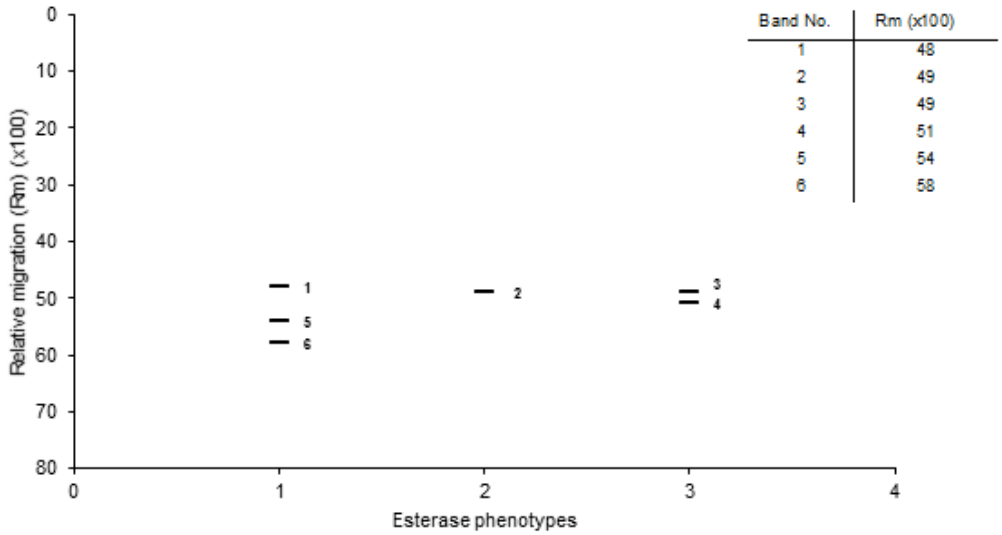


Figure 1. Schematic representation of esterase phenotypes of *Meloidogyne* populations infesting different crops in Kahramanmaraş Province, Turkey. 1: *M. javanica* (Est = J3); 2: *M. incognita* (Est = I1), and 3: *M. incognita* (Est = I2).

It appeared that the nine infested eggplant samples (8.5% of all samples) collected from Aydın Kavak (Türkoğlu) were phenotype I1, and remaining 17 infested tomato and cucumber samples (15.8% of all samples) collected from Aydın Kavak, Çiğli and Kurtepe were phenotype I2. The phenotypes, I1 and I2, are species specific and have proved to be of high diagnostic value for distinguishing *M. incognita* from other *Meloidogyne* spp. (Esbenshade & Triantaphyllou, 1985; Carneiro & Almeida, 2001; Carneiro et al., 2004; Cofcewicz et al., 2004; 2005; Brito et al., 2008). The phenotype I1 is the most common phenotype and has been observed in many populations of *M. incognita* infesting many different crops around the world (Esbenshade & Triantaphyllou, 1985; Fargette, 1987; Castro et al., 2003; Carneiro et al. 2004, Cofcewicz et al., 2004; 2005; Brito et al., 2008). Nonetheless, the phenotype I2 were also isolated from populations of *M. incognita* infesting several crops, including vegetables, fruit trees and agronomic crops in several countries (Castro et al., 2003; Carneiro et al. 2004; Cofcewicz et al., 2004; 2005; Brito et al., 2008). In our study phenotype I2 was found to be the dominant phenotype.

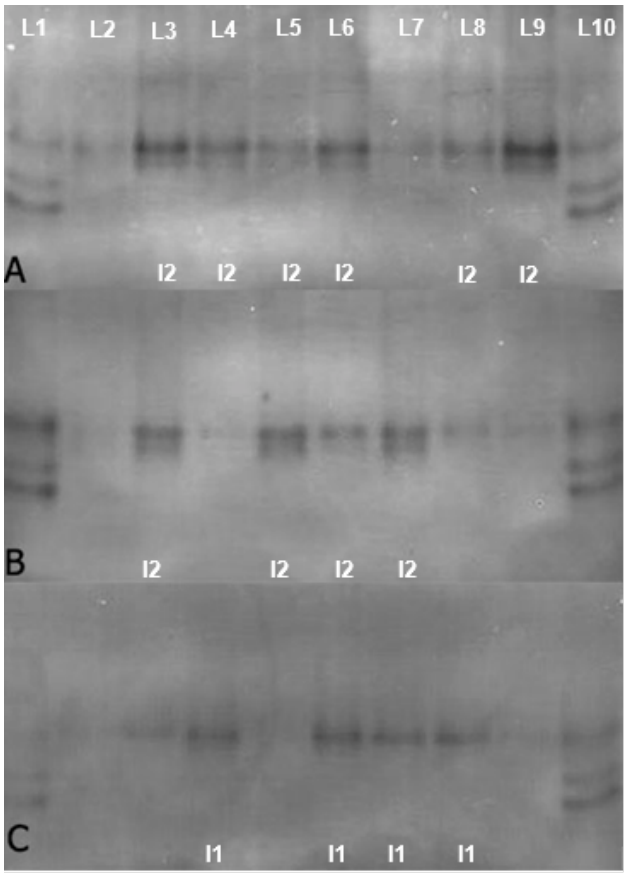


Figure 2. Esterase bands resolved from individual root-knot nematode females following electrophoresis on polyacrylamide slab gels. Lanes and 10 - standard controls, *Meloidogyne javanica* (esterase phenotype J3). Lanes 2 to 9 - *Meloidogyne incognita* from each of root-sampled vegetable hosts A) tomato (esterase phenotype I2), B) cucumber (esterase phenotype I2), and C) eggplant (esterase phenotype I1) sampled in Kahramanmaraş Province, Turkey.

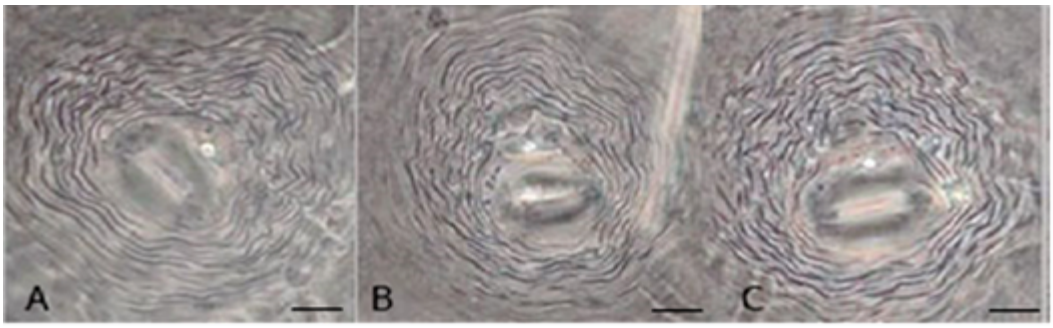


Figure 3. Perineal patterns of *Meloidogyne incognita* from A) tomato, B) cucumber, and C) eggplant sampled in Kahramanmaraş Province, Turkey (bar=20 µm).

Perineal patterns in this study were typically of *M. incognita*. *Meloidogyne incognita* perineal patterns had a moderately high squarish dorsal arch with a distinct whorl in the tail terminal area. The distinct lateral lines were absent (Figure 3).

It is difficult to distinguish all *Meloidogyne* species using perineal patterns alone due to considerable morphological variations between and within populations. However, perineal patterns are a valuable tool for supporting for other methods, such as biochemical analysis (Carneiro et al., 2004; Hernandez et al., 2004). This study also founded that the perineal patterns were valuable and corroborated the findings of the PAGE analysis.

It is believed that the excessive monocultures of pepper (*Capsicum annuum* L.) over many years has resulted the dominance of a single species of nematode (*M. incognita*) in the area sampled. It has been reported that *M. javanica* has three host races (parasitic to pepper or peanut, or non-parasitic to both) (Rammah & Hirschmann, 1990). Although there are no early reports about of *M. javanica* in this predominately pepper-growing area, we postulate that existence of non-parasitic races of *M. javanica* lead to their decline to an undetectable level. Consequently, this situation allowed *M. incognita* to dominate as a single species. Root-knot nematode races do not show any major and minor morphological differences and they can be determined only by a host test. Since race determination is not possible by morphological, cytological, biochemical criteria, it is evident that host tests need to be carried out before choosing crop rotations in a given agricultural area (Rammah & Hirschmann, 1990). Given that root-knot nematodes induce similar above ground symptoms to those caused by other pathogens and plant nutrient deficiencies, farmers should be actively informed about this pest and its impact. Before implementing chemical control, some of other management practices, including proper rotation programs, cultural control and use of resistant varieties should be implemented. Growing non-host plants, such as peanut and strawberries, or vegetable cultivars with the *Mi-1* resistant gene could reduce the pathogen incidence in this area. Solarization is also one of the important management practices that can reduce the number of nematode eggs and second stage juveniles in soil. With the root-knot nematodes found in this study occurring mostly in the vegetable-growing areas with inappropriate irrigation systems, drip or sprinkler irrigation systems should be adopted to avoid or prevent further dispersal of existing plant parasitic nematode species, especially *Meloidogyne* spp.

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