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# Distribution of the root-knot nematode *Meloidogyne* spp., in tomato greenhouses at Lattakia and Tartus Province in Syria

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**Abstract**

A survey of 35 tomato greenhouses from Syrian provinces Tartus and Lattakia revealed the presence of *Meloidogyne incognita* and *M. javanica*. In Lattakia province, *M. javanica* was the dominant species (91%) and *M. incognita* found only once (9%). In Tartus province, *M. incognita* was the most prevalent species particularly in the southern parts (76%) and *M. javanica* occurred in several locations (24%) in northern Tartus. The majority of the sampled tomato cultivars were infected with two *Meloidogyne* species; once both species were detected on the same variety.

**Keywords:** Distribution, *Meloidogyne* spp., tomato greenhouses, Tartus, Lattakia, Syria

**V**egetables are between the most important components of the daily diet all over the world and high economic value for both small and large growers (Sikora & Fernández, 2005). Within the vegetables, tomatoes (*Solanum lycopersicum* L.) occupy a very important position in terms of distribution and production. In Syria, the total area and production of tomato greenhouses and open field reaches 13.919 ha with a total production of 633,483 tons (Anonymous, 2010).

Tomato crops can suffer from abiotic stress such as drought and salinity as well as from biotic stress caused by fungi, bacteria and nematodes (Sasser *et al*., 1980). Root-knot nematodes (RKN), *Meloidogyne* spp., are major pathogens of tomatoes in fields and greenhouses worldwide (Anwar *et al*., 1991; Jones *et al*., 1991; Fourie & McDonald, 2000). RKN infected tomato plants showed yellow leaves and stunting growth. Moderate infections can cause 20-33% yield loss (Sasser, 1989; Sikora & Fernandez, 2005), while under severe infection the yield loss can rise up to 85% (Sasser, 1979; Taylor & Sasser, 1978).

At the end of 2012, nearly 100 nominal species of root-knot nematodes had been described (Karssen *et al*., 2013). Much attention is given to the so-called four major species, *viz. M. arenaria* Chitwood, *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* (Neal) Chitwood (Sasser, 1980; Moens *et al*., 2009). *Meloidogyne hapla* is distributed in temperate regions, while the other three species are common in tropical and subtropical ones but also in greenhouses independently of the climate (Sasser, 1980). The annual losses caused by these species are estimated at about $10 billion (Chitwood, 2003). In Syria, only RKN-species were reported in association with vegetables (mainly tomato and cucumber), flower crops like carnation, cotton, sugar beet and tobacco (Mamluk & Faust, 1975; Tayar, 1980; Lamberti, 1984). The presence of *M. hapla* has never been demonstrated. The widespread recognition of the four species has probably led to many cases of

misidentification (Moens *et al*., 2009). However, isozyme phenotyping and species-specific DNA protocols now allow determining RKN-isolates rapidly and with less equivocation than is possible with morphometric analysis.

To address the limited knowledge about the distribution of RKN, a survey was conducted for tomato greenhouses in the western region Tartus and Lattakia provinces of Syria. The collected RKN species were identified using traditional morphology and molecular techniques.

## Materials and Methods

**Sampling and survey distribution:** The survey was conducted during tomato growing season 2011. Fifty-two root samples were collected from 35 greenhouses located in 21 villages of the Tartus and Lattakia provinces along the Syrian coast over more than 100 km (Fig. 1). From each greenhouse, 1-2 plants (5-6-months old) showing symptoms of RKN-infection as stunting, wilting and/or yellowing were selected. Their roots were kept separately in a plastic bag along with information on the sapling site and cultivar of host (Table 1).

## Identification of isolates

**Morphology based identification:** From each sample RKN females were collected and washed galled roots. Per sample perineal patterns of five adult females were prepared and examined according to Karssen (2002).

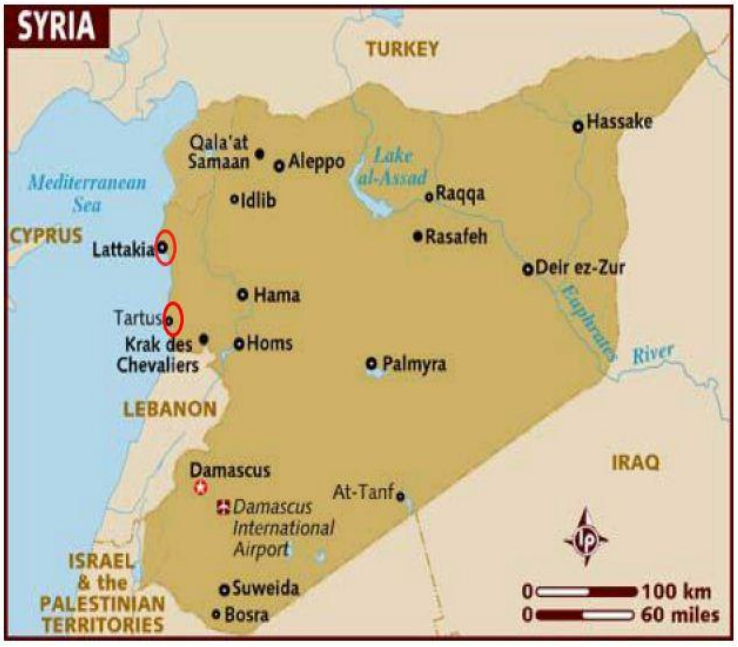


**Fig. 1.** Map of Syria showing survey sampling areas for root-knot nematodes.

## DNA based identification

**DNA extraction:** From each population, 2 egg-masses were transferred separately in a 0.5 ml tube containing 150 µl double distilled water. A plastic stick and microhomogeniser (Vibro Mixer) were used to crush and homogenise the egg masses, respectively. After a short centrifugation of the homogenate 150 µl worm lysis buffer (Holterman *et al*., 2006) was added (final concentration 200 mM NaCl, 200 mM Tris-HCl (pH 8), 1% β-mercaptoethanol and 800 µg/ml Proteinase K). After that samples were incubated for 2 hrs at 60 °C followed by 5 min at 99 °C in a thermomixer with a rotation speed of 300 t/min. The extracted DNA was stored at -20 °C for future stock use.

**Table 1. Occurrence and geographical distribution of RKN in tomato greenhouses at Tartus and Lattakia provinces.**

| Code | Govern-orate | Village | Tomato cultivar | Crop rotation | Control system | Species Identification | |
|---|---|---|---|---|---|---|---|
| | | | | | | Perineal patterns | Species-specific PCR |
| Melo35 | Lattakia | Banias | Amal Dalola | Tomato-tomato | Solarization | *M. javanica* (MJ) | MJ |
| Melo36 | Lattakia | Banias | Amal Dalola | Tomato-tomato | Liquid nematicides | MJ | MJ |
| Melo37 | Lattakia | Banias | Amal Dalola | Tomato-tomato | Solarization | MJ | MJ |
| Melo42 | Lattakia | Banias | Houda | Tomato-tomato | Solarization | *M. incognita* (MI) | MI |
| Melo47 | Lattakia | Banias | Astona | Tomato-tomato | Oxamyl | MJ | MJ |
| Melo48 | Lattakia | Banias | Astona | Tomato-tomato | Oxamyl | MJ | MJ |
| Melo51 | Lattakia | Banias | Shanon | Tomato-tomato | Liquid nematicides | MJ | MJ |
| Melo44 | Lattakia | Hrieson | Rfika | Tomato-tomato | Solarization | MJ | MJ |
| Melo49 | Lattakia | Jabla | Red Joulanar | Tomato-tomato | Solarization | MJ | MJ |
| Melo45 | Lattakia | Sarbion | Korfo | Tomato-tomato | Liquid nematicides | MJ | MJ |
| Melo46 | Lattakia | Sarbion | Korfo | Tomato-tomato | Liquid nematicides | MJ | MJ |
| Melo9 | Tartus | Ain Zebde | Rido Ring | Tomato-tomato | Solarization | MI | MI |
| Melo10 | Tartus | Ain Zebde | Rido Ring | Tomato-tomato | Solarization | MI | MI |
| Melo32 | Tartus | Biet Khalat | Sidra | Tomato-cucumber | Solarization | MI | MI |
| Melo33 | Tartus | Biet Khalat | Sidra | Eggplant-tomato | Solarization | MI | MI |
| Melo11 | Tartus | Daher Markaba | Kastel | Tomato-tomato | Solarization | MI | MI |
| Melo12 | Tartus | Daher Markaba | Kastel | Tomato-tomato | Solarization | MI | MI |
| Melo13 | Tartus | Daher Markaba | Houda grafted onto wild root stock | Tomato-tomato | Oxamyl | MI | MI |
| Melo31 | Tartus | Daher Safra | Astona | Tomato-tomato | Solarization | MJ | MJ |
| Melo5 | Tartus | Dwer Taha | Kastel | Tomato-tomato | Liquid nematicides | MI | MI |
| Melo6 | Tartus | Dwer Taha | Kastel | Tomato-tomato | Liquid nematicides | MJ | MJ |
| Melo1 | Tartus | Kfarfo | Super Star | Tomato-tomato | Solarization | MI | MI |
| Melo2 | Tartus | Kfarfo | Super Star | Tomato-tomato | Solarization | MI | MI |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Melo3 | Tartus | Kfarfo | Astona | Tomato-cucumber | Oxamyl | MJ | MJ |
| Melo4 | Tartus | Kfarfo | Astona | Tomato-cucumber | Oxamyl | MJ | MJ |
| Melo26 | Tartus | Kharab | Astona | Tomato-tomato | Solarization | MI | MI |
| Melo27 | Tartus | Kharab | Astona | Tomato-tomato | Solarization | MI | MI |
| Melo28 | Tartus | Kharab | Houda grafted onto wild root stock | Tomato-tomato | Liquid nematicides | MI | MI |
| Melo52 | Tartus | Kharab | Houda | Tomato-tomato | Oxamyl | MI | MI |
| Melo20 | Tartus | Khrab Markia | Red Joulanar | Tomato-tomato | Liquid nematicides | MI | MI |
| Melo21 | Tartus | Khrab Markia | Red Joulanar | Tomato-tomato | Liquid nematicides | MI | MI |
| Melo29 | Tartus | Markia | Amal Dalola | Tomato-tomato | Solarization | MI | MI |
| Melo30 | Tartus | Markia | Amal Dalola | Tomato-tomato | Solarization | MI | MI |
| Melo14 | Tartus | Rawdet El-Khrab | Kastel grafted onto wild root stock | Tomato-tomato | Solarization | MI | MI |
| Melo15 | Tartus | Rawdet El-Khrab | Kastel grafted onto wild root stock | Tomato-tomato | Solarization | MI | MI |
| Melo16 | Tartus | Safsafa | Darina | Tomato-cucumber | Solarization | MJ | MJ |
| Melo17 | Tartus | Safsafa | Darina | TTomato-cucumber | Solarization | MI | MI |
| Melo24 | Tartus | Safsafa | Super Star | Tomato-tomato | Oxamyl | MI | MI |
| Melo25 | Tartus | Safsafa | Super Star | Tomato-tomato | Oxamyl | MI | MI |
| Melo18 | Tartus | Sahl Meaar Shaker | Rido Ring | Tomato-tomato | Oxamyl | MI | MI |
| Melo19 | Tartus | Sahl Meaar Shaker | Rido Ring | Tomato-tomato | Oxamyl | MI | MI |
| Melo7 | Tartus | Taibt El-Mahdi | Houda | Tomato-tomato | Solarization | MJ | MJ |
| Melo8 | Tartus | Taibt El-Mahdi | Houda | Tomato-tomato | Solarization | MI | MI |
| Melo38 | Tartus | Taibt El-Mahdi | Afamia | Tomato-cucumber | Liquid nematicides | MJ | MJ |
| Melo39 | Tartus | Taibt El-Mahdi | Afamia | Tomato-cucumber | Solarization | MJ | MJ |
| Melo40 | Tartus | Tel Snon | Kortoba | Tomato-tomato | Solarization | MJ | MJ |
| Melo41 | Tartus | Tel Snon | Kortoba | Tomato-tomato | Solarization | MJ | MJ |
| Melo43 | Tartus | Teraa | Astona | Tomato-tomato | Liquid nematicides | MI | MI |
| Melo22 | Tartus | Yahmour | Astona | Tomato-tomato | Solarization | MI | MI |
| Melo23 | Tartus | Yahmour | Astona | Tomato-tomato | Solarization | MI | MI |
| Melo34 | Tartus | Zyaida | Amal Dalola | Tomato-tomato | Solarization | MI | MI |

**Checking the DNA quality:** To control the quality of each DNA extract the rDNA-ITS region was amplified by adding 1 µl of the extracted DNA to a PCR reaction mixture containing 23 µl dd$H_2O$, 25 µl Dream Taq PCR Master Mix (2×) (Fermentas Life Sciences, Germany), 1 µM forward primer 5'-CGT AAC AAG GTA GCT GTA G-3'and 1 µM reverse primer 5'-TCC TCC GCT AAA TGA TAT G-3' (Ferris *et al*., 1993). The PCR-programme was as follows: initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 49 °C for 45 s and 72 °C for 60 s. An additional amplification step at 72 °C for 8 min. After electrophoresis of 5 µl PCR product in a 1.5% TAE buffered agarose gel (1 h, 100 V) the gel was stained in an ethidium bromide bath (1 mg/l) for 30 min and photographed under UV light. In case of a positive result the DNA extract was used for species identification using species-specific primers.

**Species identification by species-specific primer sets:** From each DNA extract amplifications were done by adding 1 µl crude DNA to the PCR and in that mixture primers were replaced by 1 µM of each of the forward and reverse species-specific primers to detect *M. hapla*, *M. arenaria*, *M. arenaria*, *M. javanica*, *M. chitwoodi*, *M. enterolobii* and *M. fallax* (Zijlstra *et al*., 2000; Wishart *et al*., 2002; Tigano *et al*., 2010). Negative controls did not contain template DNA; positive controls were run with DNA of the target species (Table 2). Following all amplifications, electrophoresis was carried out in a 1.5% TAE buffered agarose gel (40 min, 100 V), stained in an ethidium bromide bath (1 mg $l^{-1}$) for 30 min and photographed under UV light.

**Table 2. Primer codes used for identification of *Meloidogyne* species their sequences, amplification length and sources.**

| Species | Code | Primer sequence 5'- 3' | Amplicon length bp | Reference |
|---|---|---|---|---|
| *M. arenaria* | Far | TCG GCG ATA GAG GTA AAT GAC | 420 | Zijlstra *et al*., 2000 |
| | Rar | TCG GCG ATA GAC ACT ACA ACT | | |
| *M. incognita* | Finc | CTC TGC CCA ATG AGC TGT CC | 1200 | Zijlstra *et al*., 2000 |
| | Rinc | CTC TGC CCT CAC ATT AAG | | |
| *M. javanica* | Fjav | GGT GCG CGA TTG AAC TGA GC | 670 | Zijlstra *et al*., 2000 |
| | Rjav | CAG GCC CTT CAG TGG AAC TAT AC | | |
| *M. enterolobii* | MK7-F | GAT CAG AGG CGG GCG CAT TGC GA | 520 | Tigano *et al*., 2010 |
| | MK7-R | CGA ACT CGC TCG AAC TCG AC | | |
| *M. hapla*, *M. fallax* and *M. chitwoodi* | JMV1 | GGA TGG CGT GCT TTC AAC | *M. hapla*: 440 *M. fallax*: 670 *M. chitwoodi*: 540 | Wishart *et al*., 2002 |
| | JMV2 | TTT CCC CTT ATG ATG TTT ACC C | | |
| | JMV hapla | AAA AAT CCC CTC GAA AAA TCC ACC | | |

## RESULTS

**Morphology based identification:** Only two RKN species viz., *M. incognita* (62% of the positive samples) and *M. javanica* (38%), were identified on the basis of their perineal pattern (Table 1). In Lattakia province, *M. javanica* was the dominant species (91%) and *M. incognita* found in only one greenhouse (9%). In Tartus province, *M. incognita* was the most common (76%) particularly in the south, while *M. javanica* occurred in several locations (24%) in the northern side of Tartus closer to the southern side of Lattakia (Fig. 2).

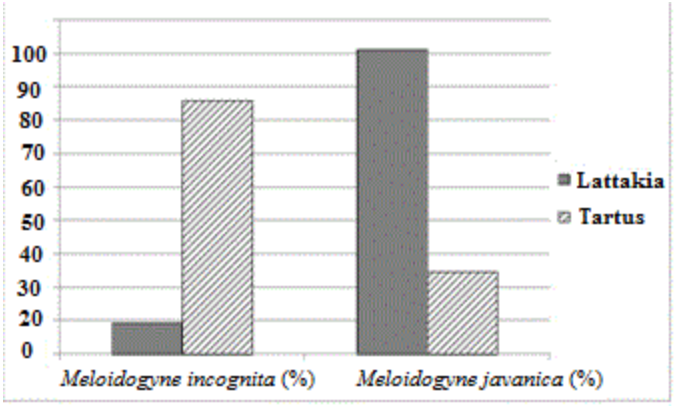


**Fig. 2.** Frequency distribution of *Meloidogyne incognita* (MI) and *M. javanica* (MJ) in Lattakia and Tartus provinces.

No link was observed between the tomato cultivars and the presence/absence of the *Meloidogyne* species. The cultivars Amal Dalola, Astona, Kastel, Red Joulanar, Darina and Houda were infected by both *M. javanica* and *M. incognita* at different locations (Table 1). In one location, i.e. Safsafa, the two species were detected in one greenhouse and infecting the same variety Darina.

**DNA based identification:** Amplification of the rDNA-ITS region was successful for all samples, and yielded a single band with an expected fragment size of 600 bp (Fig. 3) (Ferris *et al*., 1993). No PCR products were obtained in the negative control without nematode DNA template. Similarly, the PCR using species-specific primers was successful for all positive samples. PCR with species-specific primers for *M. incognita* yielded an expected fragment of 1200 bp (Zijlstra *et al*., 2000) for 32 samples (Fig. 4). When using the species-specific primerset for *M. javanica*, an expected fragment of 670 bp was obtained (Zijlstra *et al*., 2000) for 20 samples (Fig. 5). The PCR using species-specific primers not detected *M. hapla*, *M. arenaria*, *M. chitwoodi*, *M. enterolobii* or *M. fallax*. For each isolate, the DNA based identification was same results as morphological.

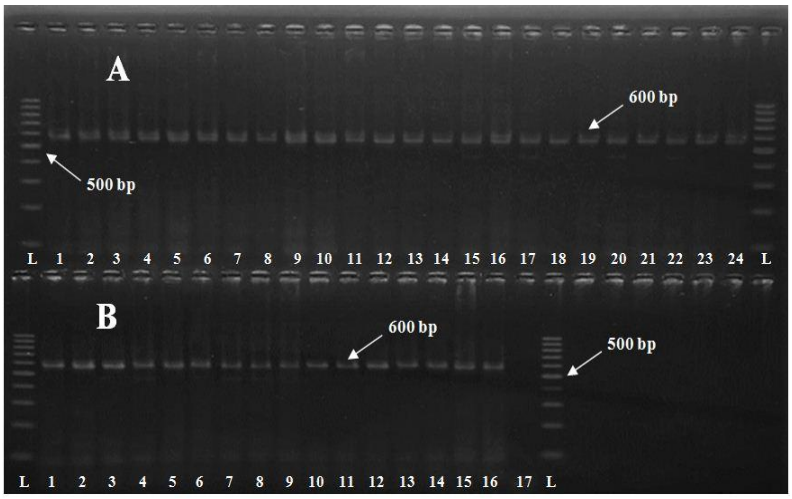


**Fig. 3.** Results of the universal PCR of ITS region (rDNA) of selected *Meloidogyne* isolates in Lattakia and Tartus provinces (Table 1). A: 1-24 and B: 1-15: *Meloidogyne* isolates, 16: positive control (Ferris *et al*., 1993), 17: negative control, L: 100 bp DNA ladder (Fermentas Life Sciences).

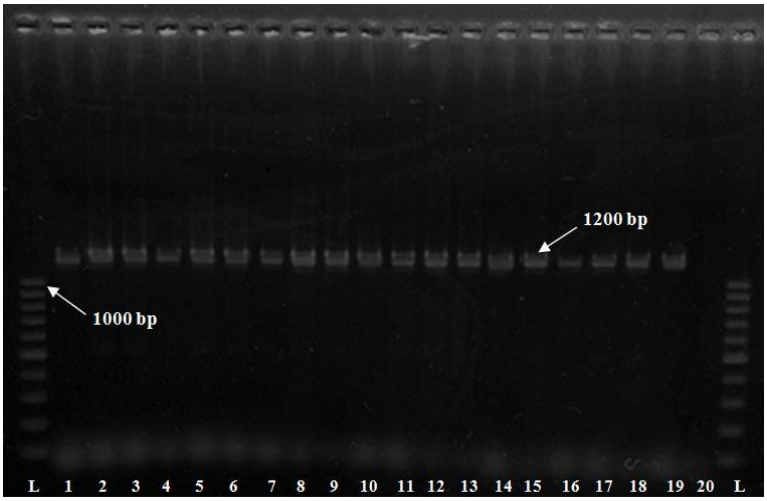


**Fig. 4.** Results of the species-specific PCR amplification for selected *M. incognita* isolates in Lattakia and Tartus provinces (Table 1). L: 100 bp DNA ladder (Fermentas Life Sciences), samples from Lattakia province: 1: Melo46. Samples from Tartus province: 2: Melo1, 3: Melo10, 4: Melo11, 5: Melo12, 6: Melo13, 7: Melo17, 8: Melo21, 9: Melo22, 10: Melo23, 11: Melo24, 12: Melo25, 13: Melo26, 14: Melo28, 15: Melo30, 16: Melo35, 17: Melo36, 18: Melo47, 19: positive control, 20: Negative control.

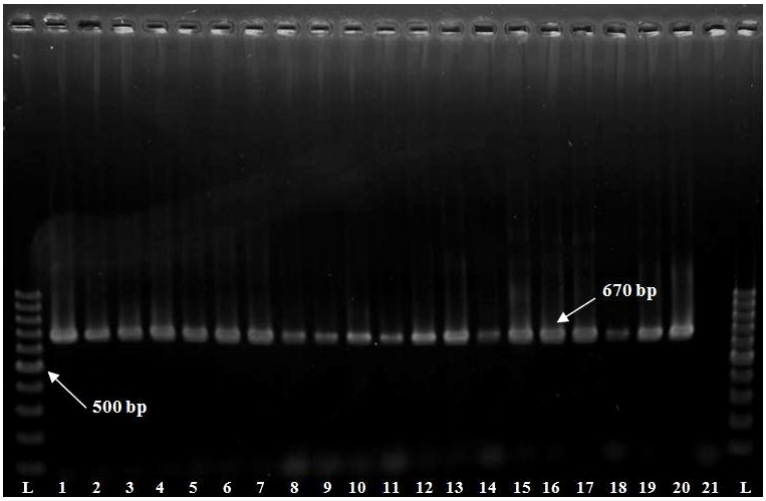


**Fig. 5.** Results of the species-specific PCR amplification for some selected *M. javanica* isolates in both Lattakia and Tartus provinces (Table 1). L: 100 bp DNA ladder (Fermentas Life Sciences). Samples from Lattakia province: 1: Melo38, 2: Melo40, 3: Melo41, 4: Melo48, 5: Melo49, 6: Melo50, 7: Melo52, 8: Melo53, 9: Melo54, 10: Melo57. Samples from Tartus province: 11: Melo3, 12: Melo4, 13: Melo6, 14: Melo7, 15: Melo16, 16: Melo31, 17: Melo42, 18: Melo43, 19: Melo44, 20: positive control, 21: Negative control.

## Discussion

The results of this survey confirm earlier reports on the dominant presence of *M. incognita* and *M. javanica* in the major tomato growing areas in Syria. Both species are generally considered to be very important, because they cause severe damage and are distributed worldwide in tropical regions (Sikora & Fernández, 2005). *Meloidogyne javanica* was the most widely distributed species in Lattakia and in the northern parts of Tartus. In the latter province, *M. incognita* was the most common RKN-species. *Meloidogyne arenaria* was never detected.

Lamberti (1984) reported similar results for *M. javanica*, but reported also the dominant distribution of *M. incognita* on roots of carnation and cucumber in Tartus, particularly in the southern parts. The latter author also found *M. arenaria* associated with *M. javanica* in a greenhouse in El-Hannadi (Lattakia). Earlier reports mentioned the presence of the 3 RKN-species on cotton and sugar beet in various districts (Mamluk & Faust, 1975). Tayar (1980) attributed losses in tobacco caused by *M. javanica*.

Both identification methods we used lead to the same result. However, the traditional identification based on the morphology of the perineal pattern was time consuming and was only possible when adult females were available. This inconvenience was reported by several authors (Hartman & Sasser, 1985; Tesařová *et al*., 2003). However, the molecular method using species-specific primers was easy to run and clear to interpret. Its accuracy and sensitivity allow for a reliable diagnosis (Tesařová *et al*., 2003). The potential to use a molecular approach even without a morphological confirmation makes this technique interesting for diagnostic laboratories with less experience for morphological identifications.

Whenever RKN were detected on tomato, the infected plants appeared in patches, were stunted and their yields would visibly be low. None of the cvs Amal Dalola, Astona, Darina, Red Joulanar, Kastel and Houda were resistant for both *Meloidogyne* spp. Most frequently, *M. incognita* and *M. javanica* were found separately on individual plants of one variety; only once both species were found on the same plant Darina in Safsafa. Situated close to the Mediterranean Sea, Tartus and Lattakia exhibit a very mild climate in winter. That explains the concentration of greenhouses for vegetable production in these areas. These crops (e.g., tomato, cucumber, eggplant) were favourable hosts for RKN and partially explain the abundance of RKN in this area. The spread of RKN in the region might further be ascribed to the common use of contaminated agricultural equipment. The high densities of RKN in soil probably increase the synergistic interactions with other root and vascular pathogens, and increase damage and crop losses (Khan, 1993).

Maintaining RKN-population densities below damaging levels can only be realised with appropriate management strategies. If crop rotation is not an option because of the susceptibility of most of the crops, the use of resistant cultivars should certainly be examined and eventually propagated. Resistance is the best option for nematode management; when available due to cost effectiveness, typically compatible with other management tactics and environmentally benign (Starr *et al*., 2002). The screening of cultivars for resistance to *M. incognita* and *M. javanica* should be initiated with high priority and using local populations. The use of physical control methods might be another route for reducing the impact of RKN. In the tomato producing areas of Syria, high temperatures prevail for long periods during the summer season; they can be used to disinfest the greenhouse soil (Sikora & Fernández, 2005). However, the conditions for a successful solarisation have not been determined yet and farmers are unaware of the benefits. The survey demonstrated that RKN were widely distributed and plays a prominent role for tomato production in the coastal areas of Syria.

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