# Incidence of Plant-Parasitic Nematodes Associated with Olive Planting Stocks at Nurseries in northern Iran

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## Abstract

*Nematode were determined in 189 soil and root samples collected from 18 olive nurseries in Golestan province (northern Iran), between 2004 and 2008. which contain eight species belonging to eight grnera The important nematodes detected, in order of decreasing frequency of infestation (percentage of samples), were Aphelenchus avenae, Meloidogyne javanica, Irantylenchus sp., Pratylenchus thornei, Helicotylenchus pseudorobustus, Boleodorus thylactus, Psilenchus hilarulus and Merlinius brevidens. No disease symptoms were noted on aboveground organs of infected plants. However, population densities of Pratylenchus and Meloidogyne spp. were at potentially damaging levels in most of the olive nurseries surveyed. Large numbers of egg masses were present within the galled root tissues that might contribute to secondary infections.*

**Keywords**: olive, *Verticillium dahliae*, defoliating and non-defoliating pathotypes, resistance

## *1. Introduction*

Olive (*Olea europaea* L.), an evergreen tree native to western Asia, is extensively grown in the Mediterranean Basin, the subtropical regions of Australia, southern Africa, and North and South America. Some 750 million trees are grown on approximately 8.5 million ha, of which about 97% are in Mediterranean countries (Nico, *et al*., 2002). Olive is the most important and traditionol woody crop that cultivated over a large areas in Iran. Olive cultivation has expanded during the last decade especially in Golestan province, the northern of Iran (Fig. 1). In this province nearly 10,000 hectar of olive orchards are present, which represents about 20% of total national olive area (Anonymous, 2007). In the last decade most of new plantations in this region established with Rooghany, Zard and Mary cultivars, which are the native olive cultivars of Iran (Sanei *et al*., 2004). Commercial cultivars of olive are planted in Iran but wild olive are the important genetical sources of olive, that residue of them can be seen in the East of Golestan province (Sanei *et al.*, 2005).

Unfortunately, olive is subjected to be attacked with a variety of fungal pathogens, which affect its health, yield and its oil quality (Sanei *et al*., 2010). In order to obtain commercial 8- to 10- month-old planting stocks, plants are grown individually in 2 to 3 liters of soil in plastic bags for several months. This soil originates from either sandy soil or loamy field soil. These natural soils can be potentially infested with soilborne pathogens, including *Verticillium dahliae* Kleb. (the causal agent of Verticillium wilt) (Sanei and Nasrollahnejad, 1995; Thanassoulopoulos, 1993) and plant-parasitic nematodes. In addition, *V. dahliae* can be spread by infected planting material (Thanassoulopoulos, 1993). Thus, the use of pathogen-free planting material and uninfested soil during olive plant propagation is essential for minimizing the effects of single or concomitant infections by soilborne pathogens during the early years of olive cultivation and for preventing pathogen spread (Sanei *et al*., 2005). The damaging potential of these events to olive production was recognized by European Union (EU) legislations by mandated certification schemes for pathogentested olive trees and rootstocks (OEPP/EPPO, 1993). Several plant-parasitic nematodes have been found associated with olive trees, including *Mesocriconema xenoplax* (Raski) Loof & De Grisse (= *Criconemella xenoplax* (Raski) Luc & Raski), *Helicotylenchus* spp., *Hemicycliophora* spp., *Heterodera* spp. (Castillo *et al*., 1999), *Longidorus* spp., *Meloidogyne* spp. (Abrantes *et al*., 1992; Diab and El Eraki, 1968; Nico *et al*., 2002; Lamberti and Baines, 1969a),

*Pratylenchus* spp.( Lamberti and Baines, 1969b), *Rotylenchulus* spp. and *Xiphinema* spp. (Diab and El-Eraki, 1968; Lamberti, and Vovlas, 1993; Nico *et al*., 2002). Conversely, limited distribution was reported for some plant-parasitic nematodes, the citrus nematode *Tylenchulus semipenetrans* Cobb and the cyst-forming nematode *Heterodera mediterranea* Vovlas, Inserra, and Stone (Castillo, *et al*., 1999; Verdejo-Lucas *et al*., 1997). Nevertheless, little information is available about plant parasitic nematodes in olive nurseries in Golestan province, northern Iran (Hosseinynejad *et al*., 1996), which is a major area for olive propagation in Iran. The objectives of this study were to determine the identity, frequency, and population density of plant-parasitic nematodes in olive planting stocks in Golestan, the key olive-producing province of Iran.

## *2. Materials and Methods*

Eighteen commercial olive nurseries in Golestan province were selected for nematode surveys (Fig. 1). The plants were grown in each nursery, using that nursery's plant material and soil substrate, in standard 15-cm-diameter black plastic bags (one plant per bag) containing 2 to 3 liters of soil. Most of the planting stocks were grown out in the open, but a few were kept under a shade-cloth mesh. In all nurseries, the plants were watered non-automatically. Each sample consisted of a randomly chosen plastic bag and the included soil and olive planting stock. A total of 189 samples were collected between 2004 and 2008. The samples belonged to olive cvs. Rooghany (40), Zard (47 samples), Mission (41 samples), Bladi (21 samples), Sevillana (10 samples), Manzanilla (11 samples), and Koroneiki (19 sample). These cultivars are the most commonly grown in Golestan province. Frequency of infestation and population density were determined. Frequency of nematode infestation was calculated as the percentage of samples or nurseries in which a nematode species was found.

Nematode population densities in both root and soil were assessed for each sample. The complete root system was washed free of soil, weighed, and used to extract nematodes by maceration-centrifugation (Coolen, 1979). Additionally, when roots of a sampled plant were galled, half of the root system of that plant was used to assess the egg population density of *Meloidogyne* spp. *Meloidogyne* eggs were collected from galled roots blended in a 0.5% NaOCl solution for 4 min (Hussey and Barker, 1973). For soil population density, the nematodes were extracted from a 100-cm3 subsample of soil by centrifugal- flotation (Coolen, 1979). Population densities of nematode species in soil and root samples were calculated as the averages of the total number of nematodes recorded for those samples in which a nematode species was found. Nematodes then were fixed in 4% formaldehyde and counted. Selected nematodes were processed to glycerin by Seinhorst's method (Seinhorst, 1962) for species identification. For identification of *Meloidogyne* spp., prineal patterns of mature females were prepared for each root-knot nematode population.

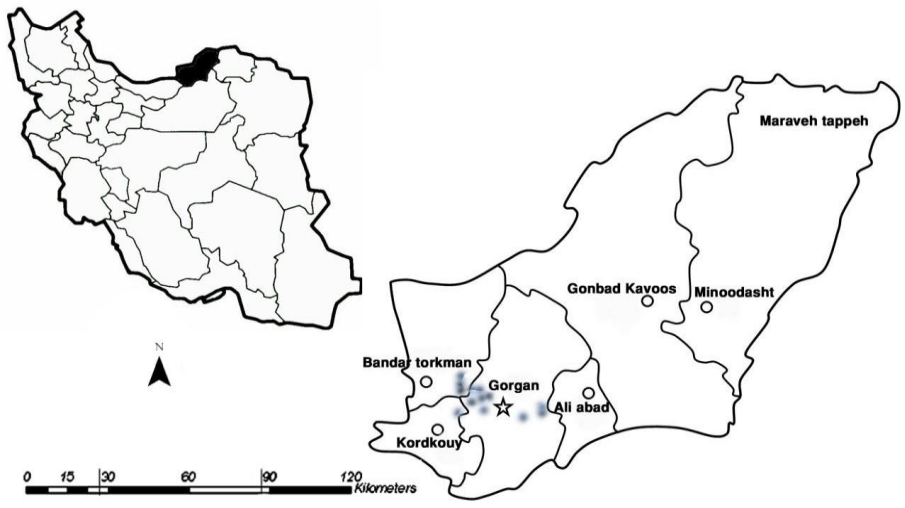


Figure 1. Iran map (left) and commercial olive nurseries surveyed (Golestan province, right) in this study.

## 3. *Results*

Eight species of plant-parasitic nematodes were found associated with olive planting stocks at nurseries in Golestan region. Plant-parasitic nematodes were detected in 122 out of the 189 root samples and in all of the soil samples.

Among species considered as potentially damaging for olive planting stocks, the genus of *Meloidogyne*, was the most important found (Table 1). Frequency of the only species of *Meloidogyne*, *Meloidogyne javanica* (Treub) Chitwood, in soil samples ranged from 22.3% (Table 1). The nematode were found infecting all the major olive cultivars, with the highest frequencies occurring in Rooghany. No disease symptoms were observed on the stems or leaves of nematode-infected planting stocks as compared with noninfected ones. However, plants infected by root-knot nematodes showed distorted feeder roots and root galls of large (6 to 8 mm) to moderate (2 to 3 mm) size. Galls occurred either singly or in clusters on the root. Other important plant-parasitic nematodes that were detected in bulk soil and roots of olive planting stocks included spiral (*Helicotylenchus* spp.), and pin nematodes (*Paratylenchus* spp.), with population densities ranging from 37 to 58 nematodes per 100 cm3 of soil and from 3 to 9 nematodes per 100 g of root (Table 1).

**Table 1.** Frequency and population densities of plant-parasitic nematodes in soil and root of olive planting stocks in Golestan province (northern Iran)[a]

| Nematode species | Frequency[b] | Nematodes/100 cm3 of soil | | | Nematodes/g of root | | |
|---|---|---|---|---|---|---|---|
| | | Mission | Rooghany | Zard | Mission | Rooghany | Zard |
| *Aphelenchus avenae* | 2.7 | 11.2±9.0 | 10.3±4.0 | 10.9±4.3 | - | - | - |
| *Boleodorus thylactus* | 2.1 | 7.3.±2.2 | 9.1.±2.7 | 8.2.±2.5 | - | - | - |
| *Helicotylenchus pseudorobustus* | 3.9 | 143.7±94.5 | 157.1±114.7 | 137.9±101.5 | - | - | - |
| *Irantylenchus* sp. | 18.6 | 38.6±7.7 | 32.6±9.7 | 39.9±8.9 | - | - | - |
| *Meloidogyne javanica* | 22.3 | 187.2±22.8 | 191.6±29.7 | 178.9±23.5 | 225.5±53.7[c] | 308.5±81.7[c] | 278.5±64.7[c] |
| *Merlinius brevidens* | 1.2 | 10.2±3.9 | 12.0±3.2 | 12.8±3.7 | - | - | - |
| *Pratylenchus thornei* | 12.8 | 42.1±5.2 | 38.7±4.2 | 37.9±5.8 | 7.3 ±3.1 | 9.7 ±2.2 | 3.2 ±2.1 |
| *Psilenchus hilarulus* | 1.9 | 27.2 ±6.1 | 22.2 ±6.7 | 24.1 ±5.3 | - | - | - |

a Data are the mean±standard error (SE) of nematodes per 100 cm3 soil and nematodes per g root in 189 soil and root samples collected in 18 olive nurseries in Golestan province (northern Iran).
b Frequency= percentage of samples in which a nematode species was found.
c Numbers indicate eggs plus second-stage juveniles collected from galled roots blended in a 0.5% NaOCl solution for 4 min.

## *4. Discussion*

The numerous plant parasitic nematodes found associated with olive are listed by Lamberti and Vovlas, (1993), which contain over 70 species belonging to 33 grnera. However, the nature of the association has not been evaluated for all species. The primary objective of this study was to determine the incidence and extent of infestation by plant-parasitic nematodes in olive nurseries in the key olive-producing province in Iran. The olive cultivars from which nematodes was isolated in Golestan province belong to eight genus and eight species and one genus (*Irantylenchus*) was new record for Iran and also add the genus *Irantylenchus* to the list of olive nematodes**.** Our survey results also indicate that more than 20% of the olive planting stocks sampled were infected singly by *M. javanica*, and approximately 10% of the stocks were co-infected by more than one of these nematodes. Little is known about the extent of and potential for damage caused by many of these species to olive. However, *Meloidogyne* spp., and *Pratylenchus* spp. can be damaging to other fruit trees (Nyczepir and Halbrendt, 1993), and reduce growth of different olive seedlings in greenhouse (Lamberti and Vovlas, 1993; Nico *et al*., 2002). Population densities of these latter nematode species in soil and olive roots ranged from moderate to high. Although damaging population thresholds for these nematodes to olive planting stocks are unknown, the population densities encountered in our survey may present a potential risk to olive planting stocks in nursery and field conditions when compared with threshold densities reported on other fruit trees (Lamberti and Baines, 1969a; Lamberti and Baines, 1969b).

Infections of cv. Rooghany by *P. thornei* significantly reduced olive shoot growth (Lamberti and Baines, 1969b). Thus, the population densities of root-lesion and root-knot nematodes found in this study have the potential to damage feeder roots, resulting in loss of vigor in young and mature olive trees as reported for other fruit trees (Nyczepir and Halbrendt, 1993). The other nematodes from this genus, *Pratylenchus thornei*, which were found in various olive planting stocks (Table 1; Nico *et al*., 2002) but not necessarily causing economic damage to olive. The result show that although no nematicide treatment threshold has been established for olive trees in this province, the nematode frequency detected in the olive cultivar tested warrant further investigations.

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