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PLANT-PARASITIC NEMATODES ASSOCIATED WITH WALNUT IN THE SANANDEJ REGION OF WEST IRAN

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Abstract: A survey of plant-parasitic nematodes associated with walnut was carried out in the Sanandej region, of the province of Kurdistan, in western Iran, during the 2011–2012 time period. After taking samples from fifty-four localities and then doing the routine laboratory work for processing, fixing, and mounting of the nematodes, twenty-one species of plant-parasitic nematodes were finally identified. Among the identified species: *Cacopaurus pestis*, *Mesocriconema xenoplax*, *Pratylenchus vulnus* and *Meloidogyne incognita* are likely to cause damage. Spiral (*Helicotylenchus crassatus*, *H. digonicus*, *H. pseudorobustus* and *H. vulgaris*), ring (*Mesocriconema antipolitanum* and *M. xenoplax*) and root-lesion nematodes (*Pratylenchus neglectus*, *P. thornei*, *P. vulnus* and *P. delattrei*) were the most predominant nematodes in the sampled area (frequencies in soil were 87.0, 77.8, and 72.2% and 46.3, 20.4 and 14.8% in root samples, respectively). In the present study, *Pratylenchus delattrei*, *Trophurus lomus*, *Paratylenchus similis*, *Geocenamus stegus*, *Helicotylenchus crassatus*, *Scutellonema brachyurus* and *Meloidogyne incognita* were reported as new species associated with walnut in Iran.

Key words: *Cacopaurus pestis*, frequency, identification, Plant-parasitic nematodes, population density, walnut

INTRODUCTION

Throughout the world, many species of plant-parasitic nematodes have been found in association with walnut (*Juglans regia* L.). Damage potential of some plant-parasitic nematode species, such as *Cacopaurus pestis*, *Mesocriconema xenoplax,* and *Pratylenchus vulnus* on walnut trees and seedlings were established (Lownsbery *et al*. 1974; 1978; Inserra and Vovlas 1981; Pinochet *et al*. 1992; Nyczepir and Halbrendet 1993; Ciancio *et al*. 1996). Only a few comprehensive surveys have been done on the identification of plant-parasitic nematodes associated with walnut in Iran (Kashi *et al*. 2004; Barooti 2006). However, some other species of plant-parasitic nematodes have been reported from walnut by different authors during miscellaneous investigations (*e.g*. Barooti 1998; Nouri *et al*. 2004; Jahanshahi Afshar *et al*. 2006; Gharakhani *et al*. 2007; 2009). A total of fifty-three species of plant-parasitic nematodes have been reported from the rhizosphere of walnut in Iran (see Ghaderi *et al*. 2012). Among them, there are some potentially important nematodes including Persian sessile (*C. pestis*), ring (especially *M. xenoplax* and *M. antipolitanum*), dagger (*Xiphinema index*, *X. pachtaicum*, *X. vuittenezi*, *X. basilgoodeyi* and *X. pyrenaicum*), root-knot (*Meloidogyne hapla*), root-lesion (*P. vulnus*, *P. thornei*, *P. neglectus*, *P. mediterraneus* and *P. pseudopratensis*), spiral (*Helicotylenchus digonicus*, *H. minzi*, *H. pseudodigonicus*, *H. pseudorobustus* and *H. vulgaris*), and pin nematodes (*Paratylenchus paraperaticus*, *P. nanus* and *P. projectus*).

Those studies about other aspects of walnut nematodes were limited and furthermore there is no available accurate information about damage levels and pathogenicity of plant-parasitic nematodes on the walnut in Iran. In a survey (Kashi *et al*. 2004), authors related high populations of *M. xenoplax* and *X. index* (maximum 292 and 265 nematodes in 100 cm^3 soil, respectively) to the decline of the walnut trees in Samen and Asadabad counties, of the province of Hamadan. The first aim of the present study was to identify plant-parasitic nematodes associated with walnut trees and seedlings in the Sanandej region. The second aim was to given some results about distribution, population densities, and the importance of these nematodes in the studied area.

MATERIALS AND METHODS

Samples were collected from the rhizosphere soil at a 5–30 cm depth, and from roots of walnut trees (53 samples) or seedlings (one sample) in orchards or from trees which were sparsely placed. The distribution map of the sampling localities is presented (Fig. 1). For each location, some subsamples were taken and then one composite sample was made from them. For each composite sam-

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Fig. 1. Distribution map of the samples taken for identification of plant-parasitic nematodes associated with walnut, in the Sanandej region, the province of Kurdistan, western Iran (coordinates: 35°_19′_02′′_N 46°_59′_56′′_E). Sample locations are shown with black triangles

ple, nematodes were extracted from the soil (500 cm^3) or roots (5 gr) by the centrifugal-flotation method. Then, the nematodes were killed and fixed by hot FP 4:1, and processed to anhydrous glycerine by the modified Seinhorst method (De Grisse 1969). The nematodes were mounted on permanent slides and examined by light microscope. Body cross sections from vermiform nematodes and perineal patterns from root-knot nematodes were made when required. All plant-parasitic nematodes, extracted from soil or root samples, were identified and counted at genus level before fixing, with the aid of Peter's counting slides under an optical dissecting microscope. Some ecological features (based on the formula developed by Norton, 1978) including absolute frequency (proportion of the infected samples to all samples), relative frequency (proportion of the absolute frequency of certain nematode genus to the sum of the absolute frequencies of all identified genera), absolute density (proportion of all individuals of a certain genus to the number of infected samples including that genus) and prominence value (absolute density multiplied to absolute frequency square for each certain nematode genus), were calculated for the identification of nematodes extracted from soil or root habitats.

RESULTS

In this survey, a total of twenty-one species of plant-parasitic nematodes were identified. However, identification of some species belonging to *Heterodera*, *Rotylenchus*, *Diphtheophora*, *Ditylenchus* and genera of the family Tylenchidae (*e.g*. *Filenchus*, *Tylenchus*, *Coslenchus*, *Boleodorus*, *Psilenchus* and *Neopsilenchus*) were not done. Results of the identified species and the calculation of ecological features for plant-plant parasitic nematodes associated with walnut in Sanandej, were shown in table 1. *Helicotylenchus* spp. predominated throughout the area (87 and 46.3% frequencies in soil and root samples, respectively), followed by *Mesocriconema* spp. (77.8 and 20.4% frequencies in soil and root samples, respectively) and *Pratylenchus* spp. (72.2 and 14.8% frequencies in soil and root samples, respectively). On the other hand, *Scutellonema brachyurus*, *Paraphelenchus basili*, *Rotylenchus* sp. and *Longidorus* sp. had the lowest frequencies (each with 1.9% frequency in soil samples).

Among the identified species, *Pratylenchus delattrei*, *Trophurus lomus*, *Paratylenchus similis*, *Geocenamus stegus*, *Helicotylenchus crassatus*, *Scutellonema brachyurus* and *Meloidogyne incognita* were found in association with walnut for the first time in Iran.

Table 1. Nematode species associated with walnut in the Sanandej region and some of their ecological features (calculated based on the formula developed by Norton, 1978)

Nematode species/character	Soil [500 cm³]				Root [5 gr]			
	Absolute frequency [%]	Relative frequency	Absolute density	Prominance value	Absolute frequency [%]	Relative frequency	Absolute density	Prominance value
Tylenchidae (*Filenchus, Tylenchus, Boleodorus, Psilenchus, Neopsilenchus*)	92.6	12.03	477.6	459.6	29.6	12.72	33.7	18.3
Ditylenchus spp.	44.4	5.77	59.5	39.7	13.0	5.56	32.9	11.8
Amplimerlinius macrurus	13.0	1.68	38.6	13.9	–	–	–	–
Geocenamus brevidens, G. rugosus, G. stegus	55.6	7.22	248.8	185.4	24.1	10.33	44.0	21.6
Trophurus lomus	11.1	1.44	66.7	22.2	–	–	–	–
Pratylenchus neglectus, P. thornei, P. vulnus, P. delattrei	72.2	9.38	356.1	302.6	14.8	6.36	134.1	51.6
Pratylenchoides ritteri	13.0	1.68	55.0	19.8	1.9	0.79	1.0	0.1
Zygotylenchus guevarai	20.4	2.65	125.2	56.5	3.7	1.59	62.5	12.0
Helicotylenchus crassatus, H. digonicus, H. pseudorobustus, H. vulgaris	87.0	11.30	616.0	574.6	46.3	19.87	50.6	34.4
Rotylenchus sp.	1.9	0.24	10.0	1.4	–	–	–	–
Scutellonema brachyurus	1.9	0.24	250.0	34.0	–	–	–	–
Heterodera sp.	3.7	0.48	22.5	4.3	–	–	–	–
Meloidogyne incognita	22.2	2.89	415.3	195.8	9.3	3.97	382.8	116.5
Cacopaurus pestis	11.1	1.44	36.7	12.2	14.8	6.36	60.6	23.3
Paratylenchus similis	25.9	3.37	193.3	98.4	5.6	2.38	13.7	3.2
Mesocriconema antipolitanum, M. xenoplax	77.8	10.10	607.3	535.6	20.4	8.74	51.4	23.2
Aphelenchus avenae	46.3	6.01	75.6	51.4	35.2	15.10	20.5	12.2
Paraphelenchus basili	1.9	0.24	20.0	2.7	–	–	–	–
Aphelenchoides subtenuis	40.7	5.29	93.0	59.4	11.1	4.77	36.7	12.2
Aprutides sp.	5.6	0.72	30.7	7.2	–	–	–	–
Diphtherophora spp.	20.4	2.65	58.6	26.5	3.7	1.59	43.0	8.3
Longidorus sp.	1.9	0.24	10.0	1.4	–	–	–	–

DISCUSSION

The population identified in the present study as *P. delattrei*, have some diagnostic characters, such as three headed annuli, empty spermatheca, the absence of males, non-differentiated post-vulval uterine sac, and a subcylindrical tail with smooth terminus. All morphological and morphometrical characters of our population are consistent with the original and other populations of *P. delattrei*, collected worldwide (Table 2). *Pratylenchus delattrei* comes close to *P. andinus* and *P. penetrans*. In *P. andinus*, the lip region with three very narrow annuli is offset by a small constriction, but in *P. delattrei*, the lip region has three distinct annuli and is continuous with the body contour. The *P. penetrans* spermatheca is full of sperm, and males are present, but in *P. delattrei*, the spermatheca is empty and males are absent.

One population collected from Galin village was consistent with *Paratylenchus labiosus* Anderson & Kimpinski (1977), morphometrically and morphologically. Brzeski (1995) synonymized four species: *P. similis* (Khan & al. 1967), *P. tateae* (Yu Wu & Townshend 1973), *P. italiensis* (Raski 1975) and *P. labiosus* Anderson & Kimpinski (1977). However, it must be kept in mind that *P. labiosus* differs from the three other species by having a full spermatheca and the presence of males (*vs.* empty spermatheca and absence of males in the three other species). This feature, though, was not considered as a stable character by Brzeski (1995) and Andrássy (2007); while other nematologists (*e.g.* Raski 1991; Siddiqi 2000) accepted *P. labiosus* as a valid species. If the validity of *P. labiosus* is correct, our population will be the first report of *P. labiosus* in Iran.

In this survey, some important migratory and sedentary endoparasitic or semi-endoparasitic nematodes viz. root lesion nematodes (*P. neglectus*, *P. thornei*, *P. delattrei* and especially *P. vulnus*), ring nematodes (*M. xenoplax* and *M. antipolitanum*), root-knot nematode (*M. incognita*) and Persian sessile nematode (*C. pestis*) were found both in the soil and roots of walnut, and sometimes with high

Table 2. Morphometric characters of the females of *P. delattrei* Luc, 1958 associated with walnut in Sanandej, west Iran and its comparison with other populations (data after from Castillo and Vovlas 2007; measurements are in µm)

Character/ Species	Present study	Luc 1958	Das & Sultan, 1979	Subramaniyan & Sivakumar, 1991	Subramaniyan & Sivakumar, 1988	Zarina & Maqbool, 1998
n	6	13	7	14	48	13
L	471 (420–592)	390–470	440–490	540 (520–570)	430 (360–490)	400 (340–460)
a	23 (21–25)	20.4–25.8	20–25	24.0 (22.0–27.4)	23.7 (20.6–26.1)	27.2 (21.6–30.7)
b	6.1 (5.2–6.9)	3.7–4.8	8.1–8.8	4.3 (4.0–4.9)	4.8 (3.6–5.4)	4.0 (3.4–5.0)
b′	4.9 (4.0–5.6)	–	–	–	3.5 (3.0–4.1)	–
c	18.7 (14.4–23.3)	18.0–22.3	18.0–22.7	19.4 (18.3–20.7)	20.5 (17.3–23.3)	18.1 (14.4–26.3)
c′	2.1 (1.6–2.7)	–	–	1.4 (1.2–1.9)	1.6 (1.3–2.1)	2.5 (1.3–3.1)
V or T	75.8 (72.2–80.0)	73–81	75–77	77 (76–79)	77 (72–79)	77 (73–86)
Stylet	17.2 (16.0–18.5)	16.5–18.0	17–18	16.5 (16–17)	17 (16–18)	15 (13–16)
Oesophagus	99 (90–105)	–	–	–	–	–
Excretory pore	53 (48–60)	66.4–76.8	–	–	–	–
Head-vulva	357 (312–448)	–	–	–	–	–
Tail length	26 (18–30)	–	–	–	–	–
Body width	19 (18–20)	–	–	–	–	–
V. body width	18 (17–19)	–	–	–	–	–
An. body width	12 (11–13)	–	–	–	–	–
Tail annuli	22 (17–25)	–	–	–	–	–

populations. Thus, it appears that these species are the likely cause of some of the drastic damage to walnut trees and seedlings in the Sanandej region. The damage potential of some of these species to walnut was clearly shown in previous studies (Thorne 1943; Pinochet *et al*. 1992; Nyczepir and Halbrendt 1993; Ciancio *et al*. 1995; 1996). The presence of *C. pestis* should not be ignored as it has been shown to be a destructive parasite of walnut in North America (Thorne 1943) or it may predispose the infested plants to dieback (Inssera and Vovlas 1981).

In the present study, pathogenicity or host range tests were not done, but high populations of some important plant-parasitic nematodes were detected in the rhizosphere or inside roots. Furthermore, galls developed by *M. incognita* were observed on the walnut roots collected from several localities. Considering these symptoms and the detection of high densities or frequencies of some important plant-parasitic nematodes, more complete studies will be required for accurately determining the importance of these nematodes in walnut production in the surveyed area. Some management strategies including the use of planting material free from nematode infection, the use of resistant rootstocks in new orchards, pre-plant applications of fumigant nematicide when replanting into an established orchard site known or suspected to be infested with important parasitic nematodes, post-plant application of nonfumigant nematicides such phenamiphos – especially against *M. xenoplax*, elimination of weeds, proper irrigation scheduling, orderly fertility treatments and reduction of other stress factors, may be helpful for reducing the yield loss of plant-parasitic nematodes on the walnut seedlings and trees in the Sanandej region.

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