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Nematodes associated with pine trees (*Pinus* spp), in the Provincial capitals of Tehran, Markazi and Qom province, Iran

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Abstract

Iran is a mountainous country with almost one-third of its land mass found in arid dessert. Consequently, trees are sparse in many Iranian provinces and those trees that do exist are often planted in urban settings or exist in small orchards. The non-native pines, *Pinus eldarica*, *P. halepensis* and *P. nigra* are widely planted throughout the major cities of Iran. Because of the aethestic and functional importance of the trees in these locations, this study was undertaken to determine if plant-parasitic nematodes known to cause significant damage to *Pinus* spp were present in urban settings. Soil samples from forest parks and urban areas with a high density of *Pinus* spp were taken from sixty-nine different locations within the provincial capital cities of Tehran, Markazi and Qom, Iran. At total of seventeen different species were identified among the three locations but none of these species identified have previously been documented to cause significant injury on pine at the levels detected. The results of the current study marks a first record of plant parasitic nematodes associated with *Pinus* spp. in Iran. Presently, there are no other reports of nematodes associated with pine trees in Iran. Based on the sampling conducted from this study, it seems unlikely that non-native pines planted in arid regions of Iran are likely to be threatened by indigenous plant-parasitic nematodes.

Keywords: Identification, morphology, plant-parasitic nematodes, *Pinus eldarica*, *Pinus halepensis*, *Pinus nigra.*

Introduction

Iran, with its arid climates and soils, is a country with low forest cover and no native pine species. As of a 2010 estimate, 7% of Iran's land is forested, of which 8% is comprised of non-native pine species. Trees are especially importance in Iran as there are only a small number that exist mainly in arid and semi-arid regions. Since 1956, Iran has imported approximately 48 fast-growing softwood species for wood and paper production (Kiaei, 2011). Conifer plantations comprised of non-native species were established to prevent erosion, counteract deforestation and improve the aesthetic qualities of public spaces (Zakeri *et al.*, 2011; Poorbabaei and Poorrahmati 2009).

The potential susceptibility of pine seedlings to various plant diseases, led to studies conducted to determine the best plant species suitable for cultivation in Iran. *Pinus pinea* L., *Pinus brutia* Ten. and *Pinus longifolia* Roxb. were found suitable for the Caspian region (Khorankeh *et al*. 2006). Several other *Pinus* species have been established in Iran and *Pinus eldarica* L. and *Pinus brutia* have been studied in nurseries in Fars Province (Zakeri *et al*., 2011).

Plant parasitic nematodes may cause significant economic damage to several plant species including those found in forest vegetation. They are, therefore,

of particular concern to countries with low forest-cover. Nematode populations have been surveyed from pine forest soils since the 1960s in North America, Europe and Asia. Seventeen genera of plant-parasitic nematodes were found associated with pines in the southeastern United States (Ruehle, 1964). Bacteria, fungi, and root-feeding nematodes were studied in a Scots pine forest near Säter, Sweden, while population sizes of soil nematode communities were studied in young pine plantations established on coal mining dumps near Cottbus, Germany (Háněl 2001, Magnusson 1983*a*; Magnusson 1983*b*). At the Changbai Mountain reserve in China, sixty genera of nematodes were found at five study sites, three of which were located in pine forests (Tong *et al*. 2009).

Plant-parasitic soil nematodes have been found to cause chlorosis, stunting, and other diseases in pine trees (Boag *et al*., 1977, Handoo *et al*., 2005; Eisenback *et al*., 1985; Fraedrich *et al*., 2003; Fraedrich *et al*., 2005; Riffle and Lucht 1966; Ruehle 1962, 1971, 1972). Host ranges of endoparasitic and ectoparasitic plant-parasitic nematodes associated with several pine species native to North America have been reported (Ruehle 1966, 1969;; Sutherland 1967). In some studies, plant-pathogenic nematodes detected in rhizosphere soils of pine trees, either did not appear to cause plant injury or their pathogenic potential was unclear (Gillespie and Adams 1962; Ruehle 1975).

The current study was conducted to determine the prevalence of plant-parasitic nematode species in 50 mixed stands of non-native, cultivated *Pinus eldarica*, *P. halepensis* and *P. nigra* trees grown in forests, parks and nurseries within three Iranian provinces. The results presented here document a wide variety of plant parasitic nematodes present in rhizosphere soils of *Pinus* spp. in Iran.

Materials and Methods

Selection of sampling sites:

Sampling sites were selected in Tehran, Qom and Markazi Provinces in Central Iran (Fig. 1). Sample site locations were recorded by global positioning system and all sites were located within suburban and urban parks and medians in capital cities of their respective provinces. The Provinces of Tehran, Qom and Markazi are classified primarily as semi-dessert with some western areas of forest steppe. Consequently, large parks and man-made forests are often planted within the boundaries of or immediately outside major cities. Samples from Tehran were collected from Chitgar Park, "Park E Eram", and Lavizan Park and few other parks. Samples from Qom (which has few large parks or man-made forests) were collected from small plots and median areas adjacent and between highways and forest. Samples from Arak in Markazi were collected from Piroozi Park, Danesshjoo Park and forest. Three species of pines, namely, *Pinus eldarica*, *P. halepensis* and *P. nigra*, were identified and located within each sampling site.

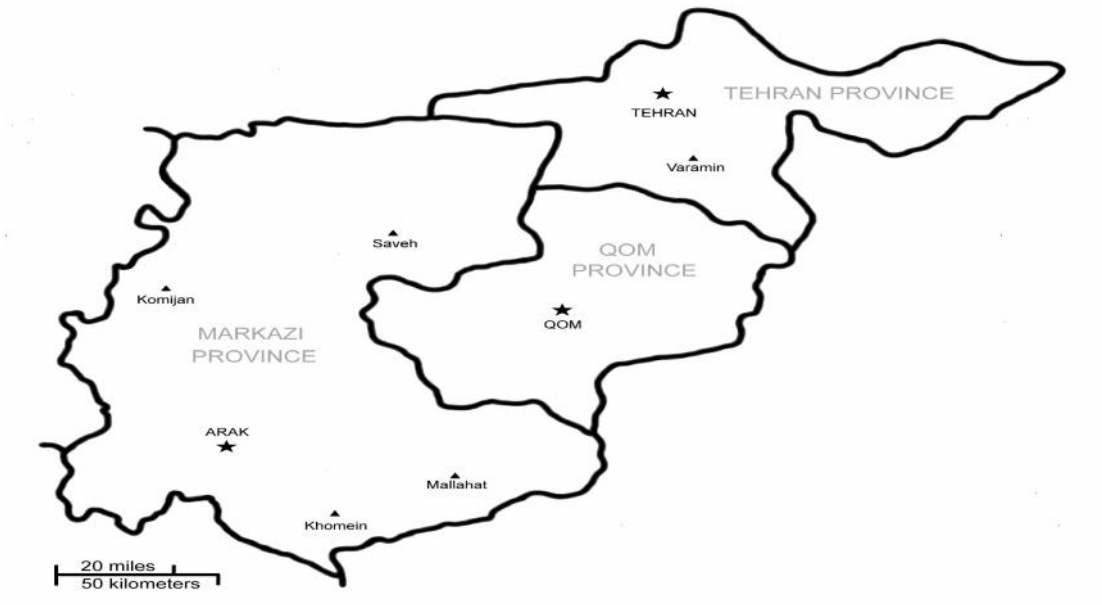

Figure Legend 1. Map of Tehran, Markazi and Qom Provinces, Iran. Twenty-one samples were taken from the national capital city of Tehran, twenty samples were taken from the provincial capital city of Arak and sixteen samples were taken from the provincial capital city of Qom.

Collection of samples:

During 2012-2015, a total of sixty-nine soil samples were collected from pine trees within the three sampling sites: twenty- four samples from Tehran, twenty samples from Qom and twenty- five samples from Markazi Province. At each site, soil samples were collected randomly from the rhizosphere region of pines at a rate of 15-22 trees per hectare.

Because of the random location of trees within each site, no standardized sampling pattern could be used, although less frequently at few sites, sampling was also done in a zig-zag pattern, assuring that each sample was taken within 1 meter of individual trees. Approximately, 1 kg soil was collected per subsample. The composite sample was thoroughly mixed and 500 g soil was processed for nematode extraction and analysis.

Nematode extraction and analysis:

Nematodes were extracted from plant tissue samples by maceration-centrifugal-flotation (Coolen, 1979) and from rhizosphere soil samples by sugar centrifugation (Jenkins, 1964). Specimens were killed with heat, fixed in FAA solution, processed to dehydrated glycerin and mounted on glass slides (De Grisse, 1969). Nematode specimens were examined through a Zeiss compound microscope with Nomarski differential interference contrast up to 1,000× magnification. Measurements were expressed as mean ± standard deviation with ranges in parentheses. Unless indicated otherwise, all measurements are expressed in micrometers (µm). Nematode species detected were compared with published original morphological and morphometric descriptions and redescriptions of the species under study (Brzeski, 1991a, 1991b; Fortuner, 1977; Geraert, 1971; Hooper, 1974; Kheiri, 1972; Krall, 1990; Loof, 1978, 1991; Sher, 1966; Siddiqi, 1970; Thorne, 1941; Thorne and Malek, 1968; Townshend and Anderson, 1976).

Results

Seventeen nematode species were identified from the three provinces collectively. Eleven species were identified from Tehran Province (Table 1), nine species from Markazi Province (Table 2) and eight species from Qom Province (Table 3). For all three Provinces, the most frequently detected plant parasitic nematode species was *Aphelenchus avenae* Bastian, 1865, in 78-85% of the total number of samples from all sites, followed by *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, in 81-69%samples. The least frequently detected plant parasitic nematode species were *Xiphinema americanum* Cobb 1913, *Xiphinema index* Thorne and Allen, 1950 and *Rotylenchus glabratus* Kankina & Teben'kova, 1980. All three sampling sites shared a similar composition of plant-parasitic nematodes associated with *Pinus* spp., however, there were certain notable exceptions.. *Pratylenchus thornei* was detected in 65% of the samples from Qom, but not found in the other provinces. Similarly, *Merlinius brevidens* (Allen, 1955) Siddiqi, 1970 (syn. *Geocenamus brevidens* (Allen) Brzeski, 1991) was detected in 70% of the samples from Tehran but not from Markazi and Qom, while *Geocenamus rogosus* (Siddiqi, 1963) Brzeski, 1991 was only detected in Markazi.

Table 1. Frequency and population densities of plant-parasitic nematodes from twenty-one soil and root samples collected from *Pinus* spp. in Tehran Province, Iran.

Nematode species	Frequency[a] %	Nematodes/100 cm^3 soil	
		Range	Mean ± SD
Aphelenchus avenae	78	20-39	34 ± 3.2
Boleodorus thylactus	67	5-15	9 ± 2.4
Ditylenchus acris	31	7-11	8 ± 2.7
Mesocriconema xenoplax	59	14-28	19 ± 4.1
Filenchus vulgaris	14	6-15	10 ± 3.3
Helicotylenchus digonicus	34	11-27	18 ± 4.9
Longidorella sp.	52	3-17	7 ± 3.3
Merlinius brevidens	70	13-38	28 ± 4.3
Pratylenchus neglectus	74	17-41	31 ± 5.1
Xiphinema index	3.5	8-12	10 ± 2.8
Xiphinema pachtaicum	27	4-15	10 ± 3.3

[a] Percentage of composite samples in which a nematode species was found.

Table 2. Frequency and population densities of plant-parasitic nematodes from twenty soil and root samples collected from *Pinus* spp. in Markazi Province, Iran.

Nematode species	Frequency[a] %	Nematodes/100 cm^3 soil	
		Range	Mean ± SD
Aphelenchus avenae	85	22-45	39 ± 4.7
Boleodorus thylactus	47	8-17	10 ± 1.8
Mesocriconema xenoplax	62	10-24	15 ± 2.5
Geocenamous rogusus	35	11-34	20 ± 1.7
Pratylenchus neglectus	81	22-48	37 ± 3.9
Pratylenchus thornei	47	17-30	15 ± 1.9
Rotylenchus glabratus	1	22	22
Xiphinema americanum	1	15	15
Xiphinema index	4.6	9-13	11 ± 3.5

[a] Percentage of composite samples in which a nematode species was found.

Table 3. Frequency and population densities of plant-parasitic nematodes from sixteen soil and root samples collected from *Pinus* spp. in Qom Province, Iran.

Nematode species	Frequency[a] %	Nematodes/100 cm^3 soil	
		Range	Mean ± SD
Aphelenchus avenae	74	14-45	37 ± 5.1
Aphelenchoides singhi	2.7	7-12	10±2.7
Boleodorus thylactus	81	11-23	14 ± 2.4
Filenchus vulgaris	29	4-17	14 ± 3.4
Helicotylenchus digonicus	72	7-22	20 ± 4.4
Pratylenchus neglectus	69	19-36	33± 4.6
Pratylenchus thornei	65	10-22	13 ± 1.2
Tylenchus filiformis	21	6-18	11 ± 3.3
Xiphinema index	2.8	6-11	9 ± 3.1

[a] Percentage of composite samples in which a nematode species was found.

Discussion

The Iranian cities sampled in the current study are located in semi-arid to arid regions, with low rolling mountains where rainfall is sparse and the majority of the local agriculture relies on irrigation. In most of these locations, forests and woodlands are uncommon and the cities of Tehran, Markazi and Qom cultivate *Pinus* and other species in order to improve the aesthetic quality of urban areas and elevate the quality of life (Edalatkhah *et al.*, 2012). Many of the locations sampled in this study were median strips and other parks where non-native pines have been intentionally established. Other areas were large forest parks, where trees are planted in rank and file, to provide a larger sense of forested wilderness that is generally absent throughout these provinces. These trees are of extremely high value and were selected to survive in arid climate with minimal amount of required management. Because of their high value, the presence and damage potential of associated plant pathogens are of concern. The results of the current study marks a first record of plant parasitic nematodes associated with *Pinus* spp. in Iran. Presently, there are no other reports of nematodes associated with pine trees in Iran.

Although pathogenicity and crop loss data for the nematodes species detected in the current study were not determined, the potential influences of the nematode species on *Pinus* spp. in Iran may be indicated from comparisons with other related reports. A number of different nematodes have been reported to cause significant damage to *Pinus* seedlings including *Paralongidorus maximus, Xiphinema americanum, Hoplolaimus galeatus, Mesocriconema xenoplax* (and *Xiphinema index* (Boag *et a*, 1977; Handoo *et al.*, 2005; Fraedrich *et al.* 2003; Fraedrich *et al.* 2005; Ruehle 1962, 1972, 1971; Sutherland and Adams, 1964; Viglierchio, 1978). In the current study, only *Xiphinema index* was identified in all locations and *Mesocriconema xenoplax* was identified from two locations. However, the maximum number of *X. index* obtained was 13 nematodes/100 cc soil. In Viglierchio (1978) study of nematode responses to *Pinus ponderosa* Laws, 100 *X. index* nematodes/150 ml soil were utilized in pathogenicity trials. Although Viglierchio did not identify the threshold at which symptoms were expressed, the populations observed from the three sampled cities in Iran were well below those used in Viglierchio's experimental trials that resulted injury to *Pinus* (taking into consideration that it is not a direct comparison between the units used in the two studies). Similarly, Viglierchio (1978) utilized 3,000 *M. xenoplax* nematodes/150 ml soil to induce damage symptoms on *P. ponderosa* and the highest number of *M. xenoplax* detected in the current study were 28 nematodes/100 cc soil.

In most reported studies, pathogenicity of soil-borne nematodes on *Pinus* spp. has been tested on seedlings than on established, mature plants. Seedlings have been used for practical reasons which include their small size, which is amenable to greenhouse and nursery experiments, and shallow root systems, which are more likely to experience observable damage due to nematodes. Plant-parasitic nematode damage typically results in stunting of seedlings, increased mortality as a result of secondary pathogens and reduced root weights (Ruehle, 1972; Mancini et al, 1983; Viglierchio, 1978). Established *Pinus* are not usually considered susceptible to soil-borne plant-parasitic nematodes although Riffle and Lucht (1966) did report an unknown *Meloidogyne* species causing decline on *Pinus ponderosa* var. *scopulorum* in New Mexico, USA and Eisenback *et al.* (1985) described *Meloidogyne pini* causing stunting, chlorosis and physiological changes to established *Pinus clausa* (Chapm.) Vasey in Georgia, USA.

Finally, *Helicotylenchus digonicus* Perry was reported to cause damage on *Pinus strobus* L. seedlings by Mancini *et al*. (1983) in the form of fewer total roots and less root weight. Similar to the work of Viglierchio (1978), Mancini *et al*. did not identify a threshold for damage and employed 125 nematodes/100 ml of soil, while the highest number of *H. digonicus* recovered from the current study were 27 nematodes/100 cc soil.

Compared to the population levels of plant-parasitic nematodes used against seedlings of *Pinus* spp in previously reported studies, it does not appear that levels of potentially pathogenic plant-parasitic nematodes are high enough to warrant concern in the urban parks and forests of Tehran, Markazi and Qom Provinces in Iran. In the current study, site locations comprised only of established stands of *Pinus* spp., so the likelihood that the low numbers of plant parasitic nematodes detected at those sites poses any threat to plant health is considered minimal.

There are approximately 120 species within the genus *Pinus* divided amongst three different groups or subgenera.

Currently, much of the reported research in nematode pathogenicity on *Pinus* spp. has focused only on a few species including *P. ponderosa, Pinus taeda* L., and *Pinus sylvestris* L.. Further research is needed to determine the effects of varied population densities of plant parasitic nematodes on different *Pinus* spp.

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