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Root lesion nematodes (*Pratylenchus* spp.) on citrus in south-west of Caspian Sea

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A survey of citrus trees in south-west of Caspian Sea was conducted to determine occurrence and population density species of root lesion nematodes. In order to surveying, 110 soil and root samples were collected during 2008 and 2009. Nematodes extracted and counted subsequently absolute and relative frequency was calculated. Four species of root lesion nematodes including *Pratylenchus jaehni*, *P. loosi*, *P. neglectus* and *P. zeae* infecting citrus trees were detected. *P. loosi* was found to be the most prevalent nematode. Its mean population density is 79 and 56 nematodes per 150 g soil and 1 g root, respectively. Above ground symptoms on heavily infected plants are not diagnostic, but include poor plant vigor and chlorosis. Below ground symptoms include poor root development and discoloration, resulting from nematode penetration and feeding on cortical cells of roots. The presence of other soilborne pathogens and saprophytic organisms would contribute to destruction of roots. Tea plant is the main host for this nematode and so far it has been reported on the tea plant and basket grass roots. However, citrus tree was introduced a new host for the nematode in Iran. *P. jaehni*, citrus lesion nematode, observed in Tonekabon, Chaloos and Noshahr orchards. The population densities ranged from 24-40 nematodes per 150 g soil and 13-27 nematodes per 1 g root. This is the first report of *P. jaehni* on citrus from regions bordered by the Caspian Sea. Results showed that *P. zeae* was least mean population and frequently relative the others.

Key words: root lesion nematode, identification, citrus, population density, Caspian Sea

Introduction

Mazandaran and Guilan located south-west of Caspian Sea and the north of Iran. These provinces enjoy a moderate, Mediterranean climate; precipitation range of Guilan province is 2089 mm and Mazandaran province is 1300 mm. Citrus is cultivated in the southern and northern parts of Iran over an area of 268000 ha which 87.07% of it is citrus trees and the rest 12.93% is seedling

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with an annual production about 4.27 million tons. Mazandaran province with the most annual production is the largest citrus producer (Anon, 2004).

Root lesion nematodes of the genus *Pratylenchus* Filipjev, 1936 rank second only to root-knot and cyst nematodes in terms of their worldwide economic impact on crops. This is due not only to their wide host range, but their distribution in almost every cool, temperate and tropical environment. *Pratylenchus* species are obligate biotrophic, soil inhabiting parasites and are found in all agricultural regions of the world. As migratory endoparasites, these nematodes destroy tissues of the root system, causing surface cracking and internal rotting of tubers and predisposing the parasitized tissues to secondary infections by fungi and bacteria (Castillo and Vovlas, 2007). Root lesion nematode infection is associated with leaf yellowing, which reduces plant photosynthesis and grain yield (Farsi, 1996). Also, there is a reduction in leaf size and number of leaves produced on heavily infected plants and fruit may be reduced substantially in number and quality. Diseased trees may appear lighter green or chlorotic and the seasonal growth of infected plants is less than would be expected in a healthy plant (Castillo and Vovlas, 2007). *Pratylenchus loosi* Attack debilitates not only the available feeder roots (inducing slow decline), but also damages the storage roots, thus limiting carbohydrate reserves (Gnanapragasam, 2002). Three species of lesion nematodes, *P. coffeae*, *P. brachyurus* and *P.vulnus* have been demonstrated to damage citrus. *P. coffeae* is easily the most pathogenic. It is widespread, having been reported on citrus in the USA, India, Japan, South Africa and Taiwan (Duncan, 2005). *P. brachyurus* in Florida on citrus was present in 90% of groves sampled (Tarjan and O'Bannon, 1969). To data, *P.vulnus* has been found associated with citrus in Italy and California and was shown to be capable of causing severe damage to nursery seedlings (Duncan, 2005). The citrus root lesion nematode (*Pratylenchus jaehni*) is considered the key nematodes for the citrus crop in Brazil and it is the most aggressive than *T. semipenetrans*. *Pratylenchus loosi,* root lesion nematodes of tea, is the most important damage agent in the tea orchards in north of Iran. Geographical distribution of the nematode was previously reported in Iran on some tea growth areas of Guilan province.

Results indicated that many important tea growth areas in Guilan were infested by this nematode. In addition, it seems that it has been distributed during short time (Hajieghrari *et al.,* 2005). However, Root lesion nematodes associated with citrus, particularly in south-west of Caspian Sea have not been fully investigated and documented. The objective of this research was to study on root lesion nematodes of citrus orchards, determine the species, population levels and frequency of occurrence.

Materials and methods

Major produce areas including East of Guilan and West of Mazandaran were selected for the sample of the study in 2008 and 2009. The regions selected in East of Guilan were Chaboksar, Rudsar, Langerud and in west of Mazandaran were Ramsar, Tonekabon, Chalus, Noshahr. The number of samples which were selected in each location depended on the hectarage of orchard. Each sample consisting of five to ten sub-samples, were collected at a depth of 10-40 cm around the citrus trees canopy. The samples were maintained in refrigerator at 4˚C until processing by centrifugal flotation technique. A total of 110 soil and root samples were taken. The 150 g soil samples were washed and the nematodes extracted by centrifugal flotation technique. Then they were fixed and transferred to glycerin according to the De Grisse method (1969).

Nematodes were extracted from roots by Terry method (Whitehead and Hemming, 1965). First the soil was washed from roots and the roots were chopped into 1 cm lengths and were placed on the terry. Terry tray full of water, So that, pieces root are in water flooding. 10 consecutive days of water in the trays passed through the sieve 400 Mesh. Nematodes collected and all of them were counted. In order to view and taking pictures of root lesion nematodes, attempting to stained root tissues. For staining nematodes in root was used of Southey (1970) method. First, the root pieces with a hypochlorite sodium solution made colorless. Then placed in acid fuchsin solution and it has been boiled for 30 seconds. Finally, roots were transferred to acidic glycerol, root Clear and nematode was observed red. A stereo microscope was used for counting number of *Pratylenchus*. Permanent slides were prepared from the extracted nematodes. After microscopic consideration, the useful measurements and drawings (morphological and morphometrical characters) were attained by using a drawing tube attached to the microscope and their identities confirmed with recent taxonomic keys. Major Characters used to identify the different species included: tail shape, whether the tail was smooth or crenate/ annulated, the number of head annules and type and number of lateral lines. (Handoo and Golden, 1989; Castillo and Vovlas, 2007).

The occurrence of a nematode species in samples is expressed as frequency. Absolute frequency is the percentage of times a nematode species occurs in a known number of samples, whereas relative frequency, also expressed as a percentage, is the relation between absolute frequency of a species and absolute frequency of all *pratylenchus* species. The mean population for each species in 150 g soil and 1 g root are stated.

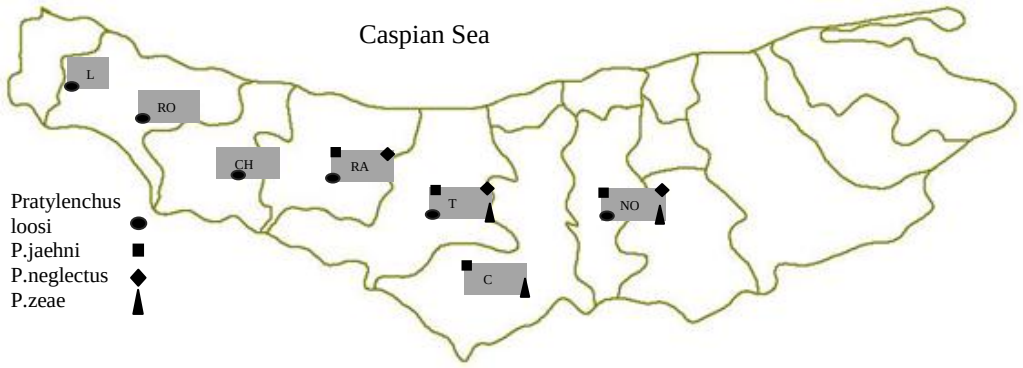

Fig 1. Distribution of *Pratylenchus* species in south-west of Caspian Sea.
L-Langerood; RO-Roodsar; CH- Chaboksar; RA-Ramsar; T-Tonekabon; C-Chalus; N-Noshahr

Results

Four species of genus *Pratylenchus* of citrus were encountered. Root lesion nematodes identified were as *pratylenchus jaehni*, *P. loosi*, *P. neglectus* and *P. zeae*. The genus had absolute frequency of 10%. *Pratylenchus loosi* Loof, 1960 was recorded as new host in soil samples of citrus. It was mean population density of 79 and 56 nematodes per 150 g soil and 1 g root respectively. It was the maximum of all species (Table 3). The *P. loosi* soil population densities ranged 43-108 and root population density ranged 32-77. In Langerood and Rudsar, citrus and tea were cultivated together. The highest population rate belongs to Langerood city with 108 and 77 nematodes per 150 g soil and 1 g root respectively. In Noshahr city mean population were 90 and 66 nematodes, which had the highest population after Langerood. Of course it has to be pointed out that in Noshahr just citrus was cultivated. Therefore it was introduced as a new host in the zone. It observed not in Chalus. It was identified based on the morphology of head, labial region rounded, with two annuli, spermatheca oval and tail tapering gradually to smooth conical tip, usually with narrowly rounded terminus (Fig 2 and 5 A) and morphometric characters. It was close to *P. coffeae*, which it can be distinguished by shape of the labial region, body and stylet length, position of the vulva and female tail shape and shape spermatheca (Castillo and Vovlas, 2007).

Fig 2. A: Entire view of female *P. loosi* B: spermatheca oval of female *P. loosi*.

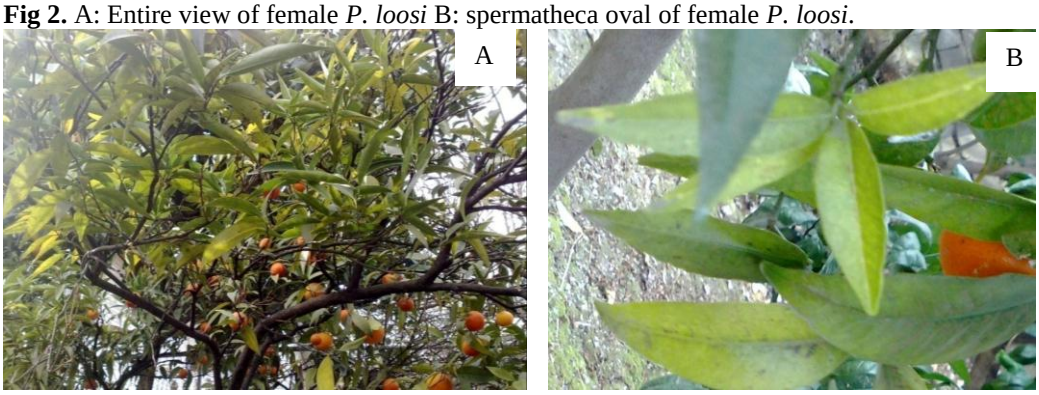

Fig 3. A: Leaf yellowing and reduction fruit size. B: Yellowing and reduction leaf size.

It is a serious pathogen of tea in Sri Lanka and other parts of the world (Seinhorst, 1977). In Iran, this species was one of the quarantine pests that for first time it were separated from the Japan imported tea slips and has been reported by Maafi (1993). Tea root lesion nematode (*Pratylenchus loosi*) as a migratory endoparasite, caused hairy roots as soon as soil temperature reaches to 15°C or above. Juvenile stages and adult females were infectious agents, the overwintering is in form of egg in soil or in feeder roots, although can overwinter in form of Juvenile or adult stages (seraji *et al.*, 2006).

Tea plant was the main host for this nematode and so far it has been reported on the tea plant and basket grass (*Oplismenus compositus*) roots in Iran (Mirghasemi and Seraji, 2010). It has been recorded in India (Sethi and Swarup, 1971) and Japan (Gotoh and Oshima, 1963) from citrus. The growth of trifoliate orange seedlings was much reduced by *P.loosi* than *Tylenchulus semipenetrans* (Ushiyama and Ogaki, 1970). Root lesion nematodes of tea, is the most important damage agent in the tea orchards, which annually causes

significant damage to tea. Infected tree growth measure strongly reduced. Due to the inability is producing new shoot the amount of product reduced strongly (Maafi *et al.,* 2003). Infected tree often showed symptoms of decline and chlorotic (Castillo and Vovlas, 2007); also in this study observed symptoms of decline tree, leaf yellowing, reduction leaf and fruit size and destroy tissues of the root system (Fig 3 and 8).

Pratylenchus jaehni Inserra, Duncan, Troccoli, Dunn, dos Santos, Kaplan and Vovlas (2001) was new recorded in soil samples of citrus in Iran. It was observed in Tonekabon, Chaloos and Noshahr cities. It was mean population density of 40 and 27 nematodes per 150 g soil and 1 g root respectively in Noshahr was the maximum among rest regions (Fig 7). Its mean population density of 34 and 23 nematodes per 150 g soil and 1 g root were the maximum after *P. loosi*. The population densities ranged from 24-40 per 150 g soil and 1 g root population density ranged 13-27. It observed not in Langerood. This species was characterized by labial region with two annuli, stylet ≤ 15 μm, female tail terminus usually hemispherical or subhemispherical and smooth and presence of males. It has been recorded only from the type locality at the Sitio das Antas farm on road SP133 near Bairro São João, county of Itápolis, in São Paulo State, Brazil, from roots of citrus (Castillo and Vovlas, 2007), the soil type is ultissol, and the annual average precipitation is 1300 mm in the zone (Inserra *et al*., 2001) And found to infest about 1 % of the nurseries and orchards. Citrus rootstocks resistance to *Pratylenchus jaehni* investigated in São Paulo State, Brazil. The Cleopatra tangerine, Swingle citrumelo, Sunki tangerine, trifoliata and Carrizo citrange were considered resistant to *P.jaehni*, while the Rangpur lime was considered susceptible (Calzavara *et al*, 2007). Morphology and morphometric characters *P. jaehni* describe that follow:

Female: Body slightly ventrally arcuate after heat relaxation and fixation. Head with two lip annuli, and a plain and smooth face with all labial sectors fused together. Stylet robust with mean length ≤ 15 µm. Dorsal pharyngeal gland opening about 2.5 µm from the stylet knobs. Hemizonid just above the excretory pore at the level of the nerve ring and two body annuli long. Hemizonion distinct and located seven to eight annuli below the hemizonid. Median bulb oval. Pharyngeal glands overlapping the intestine ventrally.

Maximum length of the pharyngeal overlap 46 µm. Lateral field marked by four incisures. Longitudinal and interrupted striae present in the central band of some specimens. Lateral field width at mid body 5.6-7.5 µm. Vulva position 78-80% of the body length. Phasmids located at about middle tail. Tail 20-25 µm long. Tail terminus usually hemispherical or subhemispherical and smooth (Fig 4 A, B and D-H and Table 1). Male: Male morphology of *P. jaehni* is similar to that of the female. However, males had shorter stylet than females

and smaller stylet knobs. The lateral field was marked by four incisures and no striae were observed in the bands of the examined specimens. The reproductive system was characterized by a single testis that does not extend to the pharyngeal glands. The bursa edge was smooth or finely crenated proximally (Fig 4 C, I and Table 1).

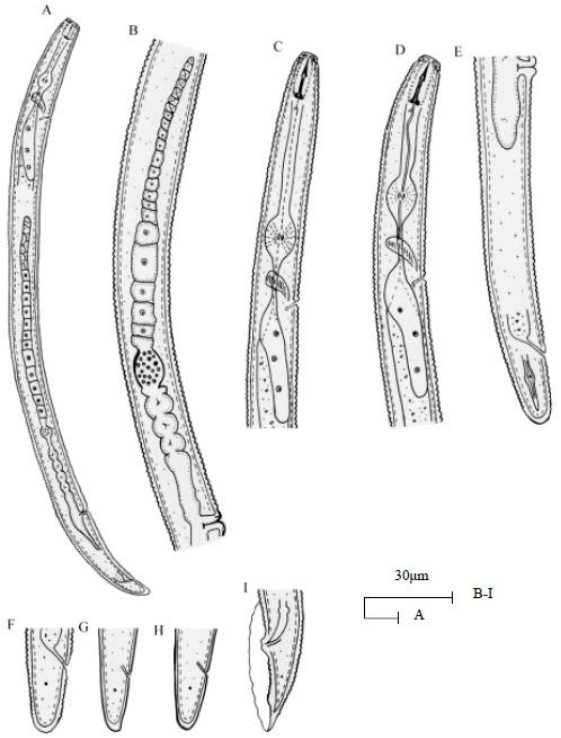

Fig. 4. A: Entire view of female. B: Female reproductive system. C: Anterior region of male. D: Anterior region of female. E: Posterior region of female. F-H: Variation in female tail shape. I: Male tail.

Pratylenchus jaehni identified according to genus *Pratylenchus* key. (Castillo and Vovlas, 2007) It is close to *P. coffeae* and *P. loosi*. It differs from *P. coffeae* and *P. loosi* by only a few morphological characters of the females. The mean values of stylet length, stylet knob height and vulva position are smaller than those in *P. coffeae* and *P. loosi*, respectively. *P. jaehni* overlap the low range values of *P. coffeae* and *P. loosi*. No morphological differences were observed among *P. jaehni* males and those of *P. coffeae* and *P. loosi*. The tail terminus was usually subhemispherical and smooth in *P. jaehni*, whereas it was commonly truncate and indented in the standard populations of *P. coffeae* and bluntly or finely pointed in *P. loosi*.

Pratylenchus neglectus (Rensch, 1924) was evidenced in soil samples of citrus. Its mean population density is 19 and 13 nematodes per 150 g soil and 1 g root respectively. Its mean population density of 27 and 15 nematodes per 150 g soil and 1 g root, in Noshahr was the maximum (Fig 7). It was described by labial region with two annuli, second annulus wider than first, stylet knobs typically indented on anterior surfaces (Castillo and Vovlas, 2007). *P. neglectus*, is a migratory endoparasite that is common to many temperate regions of the world (Williams *et al.*, 2002). It has been recorded from the type locality at Theessen near Magdeburg, Germany, from roots of rye (Duncan, 2005). It previously recorded from Iran of watermelon, peanut, soya (Pourjam *et al*., 1999a; Kheiri, 1972). It hasn't been recorded before in World of citrus (Fig 5 B).

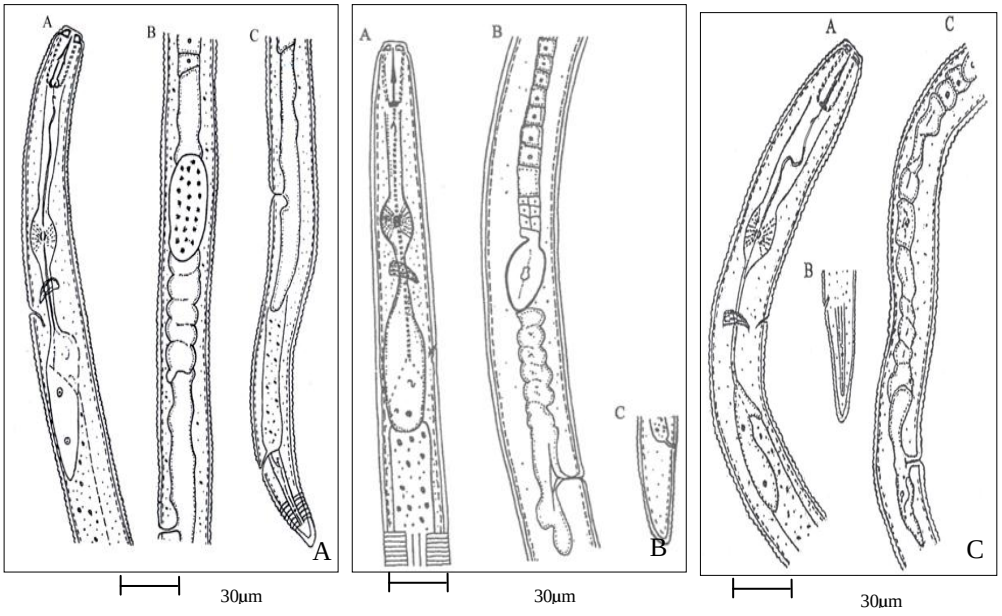

Fig. 5. A: *P. loosi*; A: Female pharyngeal region; B: Female vulval region; C: Female posterior region. B: *P.neglectus:* A: Female pharyngeal region; B: Female vulval region; C: Female tail. C: *P. zeae*; A: Female pharyngeal region; B: Female tail; C: Female vulval region.

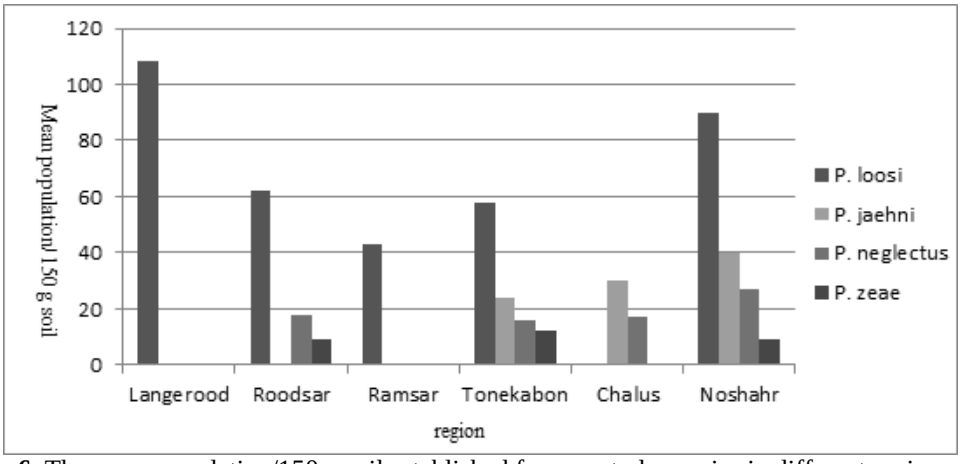

Fig. 6. The mean population/150 g soil established for nematode species in different region.

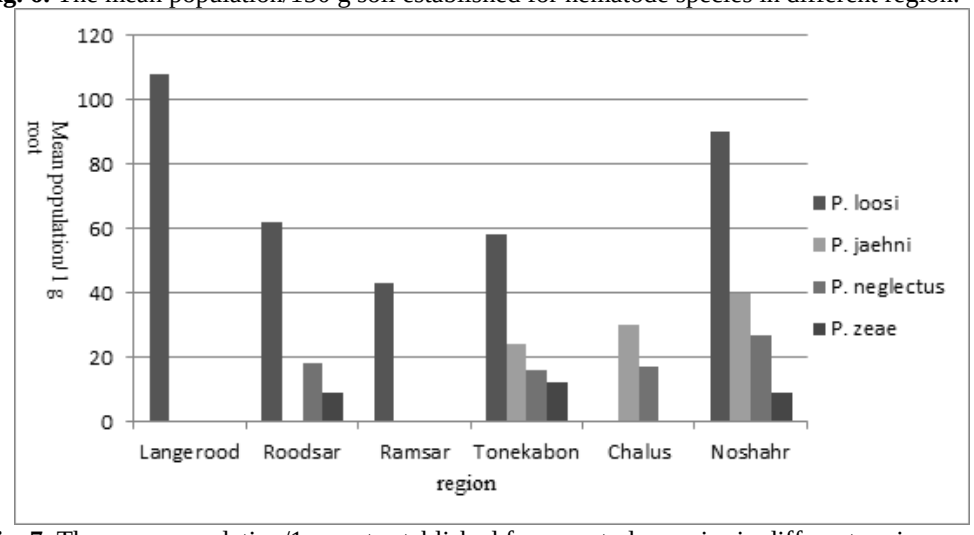

Fig. 7. The mean population/1 g root established for nematode species in different region.

Fig. 8. A: Necrotic lesions on infection roots caused by *P. loosi*. B: Lesion nematode stained in root

Pratylenchus zeae Graham, 1951 was indicated in soil samples of citrus. Its mean population density is 11 and 6 nematodes per 150 g soil and 1 g root respectively. It was characterized by labial region with three annuli, spermatheca round, small, without sperm tail conoid, terminus variable (Castillo and Vovlas, 2007). It has been recorded from the type locality at Florence, South Carolina, USA, from roots of corn, rice, sugarcane and wheat (Barker, 1998; Manzanilla-López *et al*., 2004). It is most commonly distributed throughout tropical parts of the world (MacGowan, 1981). It previously has been recorded from Iran of sunshade (Kheiri, 1972). It has been recorded from Venezuela on citrus (Crozzoli *et al*., 1998). Mean population of *p. zeae* was under rest (Fig 5C).

Discussion

Ten species of root lesion nematodes have been found associated with roots and soils of vegetables crops, but *P. loosi* (Fig 2) is the predominate species found in Guilan province and also the south-west region of Caspian Sea. At a high soil infestation density, lesion nematodes have been proven to cause significant yield loss. Damage by lesion nematodes to host crops is greater in sandy soils where water and nutrients are generally limited. In addition, lesion nematodes interact with other pathogens in causing increased damage those results in disease. The most common symptom on susceptible trees is a slow decline as the nematodes increase to very high numbers over the years. The foliage of diseased trees may appear lighter green or chlorotic (Fig 3). Once inside the root, lesion nematodes feed and move only cortical cells.

This sort of feeding does not greatly disturb the plant function. However, if population build up to high levels, the nematodes will begin to feed on tissues and plants may show visual sign of injury such as yellowing. Flowers or fruit may be reduced substantially in number and quality. The vigor of the host is reduced, and the plant may be predisposed to winter injury or other infectious diseases. The symptoms on infected roots initially are small, light to dark brown lesions (Fig 8A). These lesions tend to expand and to merge as the growing season progresses, giving the roots a discolored appearance overall. Nematode feeding causes cortical tissue disintegration and girdling (Fig 8B), resulting in the sloughing off of the epidermis and remaining cortex and a reduction in the size of the root system.

P. loosi and *P. neglectus* were the most frequently encountered species; however, mean population, distribution in the zone *P. loosi* was more than the other species. The effect of these nematodes on citrus trees in these regions in not known. Due to economical importance of *P. loosi* and its introduction as a new host for citrus in the zone, it is suggested that further investigations be

carried out on it. *P. zeae* was least mean population and frequently relative the others. Considering the importance of these species, it is suggested that scheduling more studies to assess their economic importance and management approaches. Tangerine was considered resistant to *P. jaehni* in Brazil and because this nematode observed on tangerine rootstock in north Iran. Therefore recommended that prevent of culture sensitive rootstock in this zone. Also, most species diversity is seen in the two regions Noshahr and Tonekabon that a large part of the country's citrus production is devoted to these areas. So, the purposed measures for controlling root lesion nematodes, including biological and chemical control and resistant varieties will be done. Further studies are required to determine the economic importance, biology, ecology and host range of species of root lesion nematode on citrus. Their identification using both morphological and molecular criteria may also need to be investigated, as complications may arise using the current diagnostic methods.

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