Reported some new species of plant parasitic nematodes from rhizosphere of tea plantation in Iran

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Abstract

Tea (Camellia sinensis) is one of the most important produce in Guilan province that most field is allocated to this produce after rice in this province. In order to identify the plant parasitic nematodes of Tea plantation, 340 samples of soil around the roots of Tea were collected from different parts of the Tea plantation in provinces of Guilan and Mazandaran, during the summer and fall of 2011. After extraction, killing, fixation and transferring to anhydrous glycerol, the nematodes were mounted on permanent microscopic slides and nematodes species identified by using light microscope, equipped with digital camera, based on morphological and morphometric characters using valid keys. In this study 22 species belonging 12 genera of nematodes infraorder Tylenchomorpha were identified, that before just 6 Species are reported from rhizosphere of Tea in Iran. others species are reported from rhizosphere of Tea in Iran from first reported.

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Introduction

Tea, *Camellia sinensis* (L).O.Kuntze, cultivated on 2.85 million ha, with a total production of 3.87 million ton per annum. Tea is considered as a strategic economic crop in Iran. According to FAO statistics in 2010, tea is already harvested in Iran from a surface of about 32000 ha (FAO, 2011). Tea plant is an evergreen woody perennial and grown mostly as a monoculture, provides a suitable condition for the pest to feed on and breed. It supplies plenty of food on one hand and on the other the problem of finding a suitable host for breeding purposes is much reduced. About 300 species of insect, mites and eelworms are active in tea areas but all of them are not economically important (Ghosh Hajra, 2001). Plant parasitic nematodes of tea which are found in the soil affect the root system of the plant. There are 40 species belonging 20 genera of plant parasitic nematodes are associated with tea in different tea growing countries. But most of them have no positive evidence of pathogenicity (Sivapalan, 1972). Most of diseases of tea are of fungal origin and only a few are caused by bacteria, algae and viruses. (Willson, 1999). *Pratylenchus loosi* is a serious parasite of tea in Iran (Pourjam *et al.* 1999, Seraji, 2007).

Chandra in studies that have been done on diseases of tea following nematode root tea has been reported (Chandra, 1996):

*Rodopholus similis, Hoplolaimus columbus, Meloidogyne incognita, Meloidogyne arenaria, Meloidogyne javanica, Meloidogyne hapla, Meloidogyne brevicaudata, Meloidogyne thomasi, Paratylenchus curvatus, Rotylenchulus reniformis, Pratylenchus loosi, Pratylenchus brachyurus, Hemicriconemoides kanayensis, Helicotylenchus divystera, Helicotylenchus erthrinae, Rotylenchus sp, Tylenchorhynchus sp*

Two species of root-lesion nematodes (RLNs), *Pratylenchus loosi, Loof 1960 and P. brachyurus* (Godfrey) Godey, are known to attack tea plants in some producing countries such as Sri Lanka, Philippines, Japan, China, Bangladesh, Taiwan, Indian, Vietnam, USA and Australia (Gnanapragasm *et al.* 1993).

Although research has been done on root lesion nematode *Pratylenchus loosi* in Iran, no comprehensive study has been done on identifying nematodes this product. For this reason, this study aims to investigate the tea plant parasitic nematodes were implemented in tea gardens of Iran.

Materials and methods

Field experiment

During July to October 2011, 340 soil complex samples were collected from all peanut fields in provinces Guilan and Mazandaran of Iran. Sampling was done according to cultivated area and distribution of peanut fields. Ten soil complex samples were taken from 20 to 30 cm depth in the root zone at each site. Some tea feeder roots were also collected. Soil samples were mixed and then were taken to the laboratory.

Stages of laboratory testing

Samples for nematode analysis were stored in a refrigerator until nematode extraction. Nematodes were extracted from soil using De Grisse, 1969. Then extracted nematodes fixed and transferred to glycerin (De Grisse, 1969). Then microscopic slides were prepared by using glycerin and paraffin rings. Nematodes identified by morphological and morphometrical characters and identification keys and references.

Results and discussion

In this study, 22 species of 12 genera of nematodes infraorder Tylenchomorpha were identified as follow: *Aphelenchoides asterocaudatus, Aphelenchoides bicaudatus Aphelenchoides sacchari, Aphelenchus avenae, Basiria graminophila, Criconemoides parvus, Crossonema civellae, Ditylenchus medicaginis, Ditylenchus myceliophagus, Filenchus vulgaris, Helicotylenchus digonichus, Helicotylenchus dehystera, Helicotylenchus pseudorobustus, Mesocriconema xenoplax, Mesocriconema ornatum, Paratylenchus bukowensis, Paratylenchus elachistus, Pratylenchus brachyurus, Pratylenchus loosi, Pratylenchus neglectus, Pratylenchus penetrans,*
All of the above species previously have been reported from other hosts for parasitic nematodes in Iran, are not new. Six species *Aphelenchus avenae*, *Pratylenchus loosi*, *Basiria graminogilha*, *Aphelenchoides bicaudatus*, *Filenchus vulgaris*, *Helicotylenchus dihystera* have already been reported from the rhizosphere of Tea. Other species (16) are going to report for the first time on Tea rhizosphere and are new in Iran.

**Fig. 1.** *Mesocriconema xenoplax*; A: Anterior end of female, B: Head shape, C: posterior end of female.

*Mesocriconema ornatum*; A: Anterior end of female, B: Head shape, C: Posterior end of female.

*Criconemoides parvus*; A: Anterior end of female, B: Tail shape.

In this study, 22 species belonging 12 genera were identified that before just 6 Species were reported from rhizosphere of Tea in Iran. Other species (16 Species) are going to report from rhizosphere of Tea in Iran as a first. The common species, have not described in this article, but other species that are serious parasite have been fully described.

According to the damage-causing species *Pratylenchus brachyurus* after *P.loosi* in world and
this species identified in the first time in Tea gardens of Iran is a very important.

**Pratylenchus brachyurus, (Godfrey, 1929)**

Filipjev&Schuurmans Stekhoven, 1941

Measurements female (n=5), L= 671±45 (556-883), a= 21.6±0.15 (20-24), b= 6.1±2.9 (4-6.7), b’= 7.5±2.7 (5.1-9.9), C= 19.9±2.3 (11.1-23), C’= 2.2±0.6 (2-2.7), V= 85.6±3.3 (80.8-88), Stylet= 18.8±0.2 (18-18.5), Oeso= 89.8±5.3 (86-93), Overlapping= 22.3±0.07 (22-22.8), H-V= 580±295 (370-700), V-a= 55±9.3 (48-62), B.W.= 35±5.6 (20-33), A. B. W= 15.9±2.2 (14-17), Tail= 35±2.3 (31-40).

**Fig. 2. Crossnema civellae; A: Anterior end of female, B: Reproduction system, C: Tail shapes, D: Posterior end of female.**

Female: Lateral field marked by four incisures. Lip region angular, set off from body, bears two distinct annules. Stylet knobs rounded, little variation in shape basal knobs large, round. Outer margins of heavily sclerotized labial framework extend into body laterally about one body annule. Spear-guiding apparatus extends posteriorly from basal plate about five body annules. Orifice of dorsal esophageal gland about 2 μm behind spear base. Hemizonid two or three annules long, located one to five annules in front of excretory pore. Ovary does not extend to esophageal gland, consists of single row of oocytes. Posterior uterine branch short, about width of body at vulva. Spermaphere inconspicuous, not functiona Tail broadly conoid, smooth, with broadly rounded, truncate or spatulate tip, 13-24 ventral annuli. Pharyngeal glands overlapping intestine ventrally.

This species is close to *P. japonicus* Ryss,1988 and *P. neobrachyurus* Siddiqi, 1994 from which it differs by body and stylet length, shape of labial region, shape of stylet knobs, position of the vulva and female tail shape (Castillo et al., 2007). Males were not observed in this study. The species were identified and collected in city Rudsar. (Figure 4).

**Pratylenchus neglectus Stechoven.&Filipjev, 1941(Rensch, 1924)**

Measurements female (n= 10), L= 506±25 (411-713), a= 32.5±2.5 (24.2-36), b= 4.3±0.53 (3.9-7.1), b’= 3.3±0.7 (3-5.2), c= 20.3±2.9 (16.3-26.2), c’= 3±0.7 (2.7-7.3), V= 83.7±0.99 (80.4-88), Stylet= 17.1±0.38 (16.7-18), Oeso= 118.4±15.6 (100-140), Overlapping= 36.9±3.2 (25-45), H-V= 425.9±15.2 (402-629), V-a= 55.5±7 (44.4-69.3), B.W.= 15.6±0.74 (11.3-16.2), A. B. W= 8.3±0.95 (6-10.5), Tail= 25±4.3 (22.1-28).

A parthenogenetic species characterized by great variation in body length and width, tail shape and thickness and early maturity of adults which is sometimes completed during the final ecdysis. Feeding, mature females extracted from roots are often markedly more robust than females obtained from the rhizosphere, which become more linear when relaxed (Castello et al., 2007). Labial region with two annuli, second annulus wider than first, stylet knobs typically indented on anterior surfaces, post-vulval uterine sac less than or equal to body diam, tail variable in shape usually conoid with little curvature of ventral surface and tail terminus without annulation, usually rounded, but may be obliquely truncate or slightly digitate.

**Fig. 4. Crossnema civellae; A: Anterior end of female, B: Reproduction system, C: Tail shapes, D: Posterior end of female.**

Lip region with two annuli. Stylet knobs indented anteriorly Lateral fields with four incisures, Tail variable in shape, usually conoid with little curvature of ventral surface and tail terminus with five annules long, located one to five annules in front of excretory pore. Ovary does not extend to esophageal gland, consists of single row of oocytes. Posterior uterine branch short, about width of body at vulva. Spermaphere inconspicuous, not functiona Tail broadly conoid, smooth, with broadly rounded, truncate or spatulate tip, 13-24 ventral annuli. Pharyngeal glands overlapping intestine ventrally.

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The species is close to *P. panamaensis* Siddiqi, Dabur & Bajaj, 1991 and *P. roseus* Zarina & Maqbool, 1998 and *P. yamagutii* Minagawa, 1991 from which it differs by body and stylet length, position of the vulva, tail shape and tail terminus (Castello *et al.*, 2007). Males were not observed in this study. The species were identified and collected in city Chaboksar. (Figure 4).

**Fig. 3.** *Paratylenchus bukowinensis*; A: Anterior regions of female, B: Anterior regions of male, C: Tail shape of female.

*Paratylenchus elachistus*; A: General view of female, B: Anterior regions of female, C: Anterior regions of male, D: Tail shape of male, E: Tail shape of female.

*Pratylenchus penetrans*, (cobb, 1917) Filipjev & Schuurmanns Stekoven, 1941

Measurements female (*n*= 8), *l*= 576±64 (466-652), *a*= 31.6±2.9 (27.5-36.5), *b*= 5.3±0.7 (3.9-6.1), *b*= 4.2±0.34 (3.9-4.7), *c*= 24.9±2.5 (21.4-28.3), *c*= 1.7±2.8 (1.2-2.2), *V*= 79.2±1.8 (76.6-81.1), Stylet= 16.1±0.61 (15.9-17.7).

Measurements male (*n*= 3), *l*= 526±18 (499-513), *a*= 24.3±2.3 (21-25), *b*= 4.6±0.3 (4.2-4.8), *Stylet= 15.3±0.5 (14.3-15.8), *Oeso*= 115±6 (110-115), Overlapping= 20±0.5 (29-21), Guber.= 5±0.3 (4-6), Spicule= 11±0.76 (10-12).

Female: Body moderately slender, almost straight.
when heat-relaxed Lateral field marked by four incisures. Lip region slightly set off from head bears three annules. Outer margins of heavily sclerotized labial framework extend into the body about one body annule. Spear about 18µm long, well developed, broadly rounded basal knobs. Pharynx overlapping intestine ventrally. Spermatheca round, oviduct cellular about twice as long as spermatheca, uterus about as long as spermatheca. Posterior uterine branch short, about equal to the width of body at vulva. Tail generally rounded, tip smooth, with 15-27 annuli on ventral surface.

Fig. 4. *Pratylenchus brachyurus*; A: Anterior end of female, B: Reproduction system, C: Head shape, D: Lateral field. *Pratylenchus penetrans*; A: Anterior end of female, B: Head shape, C: Tail shape, Spicule and Bursa of male D-E: Tail shapes of female, F: Reproduction system. *Pratylenchus neglectus*; A: Anterior end of female, B: Reproduction system and end of body female.

Male: Similar to female and Slightly smaller than female. Single outstretched testis consists of spermatocytes arranged in double row. Phasmids slightly posterior to middle of tail, extend only slightly into bursa. Spicules slender, with well-marked manubria and ventrally arcuate shafts. gubernaculum simple bursa irregularly crenate along margin, enveloping tail tip.

*Pratylenchus penetrans* is characterised by: labial region slightly offset, low, flat in front, with rounded outer margins, with three annuli, pharynx overlapping intestine ventrally in a lobe ca 1.5 body diam. long, post-vulval uterine sac short, undifferentiated, and tail generally rounded, tip smooth. It can be distinguished from closely related species *P. fallax*, Seinhorst, 1968 and *P. pseudofallax* Café-Filho & Huang, 1989 and *P. subpenetrans* Taylor & Jenkins, 1957 by body and stylet length, number of lip annuli, labial framework, position of the vulva, shape of spermatheca and tail terminus (Castello, 2007). The species were identified and
collected in city Lahijan. (Figure 4).

**Criconemoides parvus Raski, 1952**

Measurements female: \( n=8 \), \( L=334\pm33.6 \) (260-346), \( a=124\pm11 \) (113-144), \( b=3.8\pm0.28 \) (3.1-4) , \( c=57.4\pm12.4 \) (47.9-65) \( V=96.1\pm0.17 \) (95.9-96.5), \( Stylet=33.0\pm1.8 \) (30.2-36.1), \( m=75\pm6.9 \) (59.9-82)

\( \text{Oesophagus}=81.4\pm4.9 \) (74.5-78.8), \( R=153\pm4.4 \) (148-160), \( Rst=21.7\pm1.4 \) (19-23), \( Roes=45.8\pm3.8 \) (41-53), \( RV=9.4\pm0.73 \) (8-10), \( Ran=5.4\pm0.9 \) (5-7), \( Rvan=4\pm0.76 \) (3-5), \( VL/VB=0.73\pm0.03 \) (0.7-0.8)


Differential diagnosis. *Criconemoides parvus* can be easily recognised by its short body, finely crenate, fine annulation of the body, shortish stylet of <50 μm, open vulva, and rounded postvulval area (wouts, 2006). Males were not observed in this study. The species were identified and collected in city Lahijan. (Figure 1).

**Crossonema civellae (Steiner, 1949) Mehta & Raski, 1971**

Measurements female: \( n=14 \), \( L=474\pm37.6 \) (419-526), \( a=5.6\pm0.43 \) (5.1-6.1), \( b=3.7\pm0.22 \) (3.4-3.9), \( c=24\pm8 \) (20-35) , \( c'=0.4\pm0.15 \) (0.6-0.7), \( V=90\pm3 \) (87-95), \( Stylet=84\pm12 \) (76-91), \( \text{Oesophagus}=128\pm11.8 \) (111-142), \( R=45\pm13 \) (44-48), \( Rst=8\pm0.75 \) (7-9), \( Roes=12.6\pm0.52 \) (12-13), \( RV=7\pm0.63 \) (6-8), \( Ran=4.1\pm0.41 \) (4-5), \( Rvan=2.8\pm0.41 \) (2-3), \( Tail=21\pm8.2 \) (14-30), \( VL/VB=0.9\pm0.19 \) (0.7-1.2).

Female: Body annuli retrorse, more so posteriorly; each annulus bears a continuous fringe of spines; these vary from a simple digitate elongate spine to elavate ones, they are usually narrow, closely adjacent to each other circling the whole annulus; elongate in some cases extending to succeeding annuli. Head of two annuli, the first annulus wider than second, with a fringe of fine spines mostly directed forward; second annulus narrow rounded, Labial disc elevated, with six lips. Spear slender. Ovary outstretched reaching as far anterior as base of stylet. Vulva simple transverse slit, squared off; region of vulva without spines; both lips of vulva about equal in size, and at its distal margin does not extend outward as far as profile formed by adjacent annular spines. Excretory pore and anus obscure. Spermatheca offset, without sperm. Body posterior to vulva, disregarding scales, narrowing gradually to conical tail. Tail terminus surrounded by scales of last body annules.

Diagnosis. *Crossonema civellae* is characterised by its continuous fringe of short digitate spines on the body annules, blunt, knobbed, or bifurcated at the tips. *Crossonema civellae* most closely resembles *Crossonema palmatum* Siddiqi & Southey, 1962 in general morphology, morphometrics, and arrangement of spines on the postvulval region. It can be easily distinguished from this species by the continuous fringe of narrow spines along the outer edge of the body annules (wouts, 2006). *Crossonema civellae* has a worldwide distribution on cultivated plants (Mehta & Raski, 1971). Its restricted distribution in New Zealand is probably a reflection of the limited sampling of cultivated and ornamental plants (wouts, 2006). Males were not observed in this study. The species were identified and collected in cites Lahijan and Langeroud. (Figure 2).

**Mesocriconema xenoplax** (Raski, 1952) Raski & Loof, 1989

Measurements Female: \( n=12 \), \( L=516\pm58 \) (435-577), \( a=9.3\pm0.26 \) (8.9-9.6), \( b=3.7\pm0.44 \) (3.1-4.2), \( c=33.1\pm9.4 \) (31.5-49.2), \( c'=0.3\pm0.11 \) (0.5-0.7), \( V=94.2\pm1.1 \) (92.3-95.5), \( Stylet=74\pm8.5 \) (60-83.5), \( m=76\pm8.6 \) (74.3-82), \( \text{Oesophagus}=137.1\pm12.2 \) (112-
Female: Body annuli smooth to rough, retrorse. First two annuli not retrorse and narrower than succeeding, body annuli but not set off. The first head annulus emarginates laterally. Sclerotization hexaradiate with internal innervations present in four submedian sectors. Anastomosis with preceding or succeeding annules often complicates vulva pattern. Ovary extends. Anus located from end of body. Labial plates present. Submedian lobes rounded. Vulva open. Vagina sigmoid. Tail terminus rounded to rounded-conical. Mesoocriconema xenoplax can be recognised by its lip region with distinct sublateral lobes, the open vulva, S-shaped vagina, and blunt tail. It can be distinguished from M. rusticum (Micoletzky, 1915) Loof & De Grisse, 1989 by its longer body (R= about 0.6 mm v. about 0.45 mm) and stylet (about 70 μm v. 55 μm) and the S-shaped vagina (Wouts, 2006). Males were not observed in this study. The species were identified and collected in city Ramsar (Figure 1).

Mesoocriconema ornatum (Raski, 1958) Loof & De Grisse, 1989

Measurements Female: (n= 8), L= 400±31 (334-450), a= 11±1.6 (9-14), b= 3.5±0.33 (3.4-4.4), c= 31.4±10 (23-54), c‘= 0.18±0.27 (0.5-1/1), V= 92±0.73 (91.7-94.2), Stylet= 59.2±5.3 (47-63), Oesophagus= 104.4±9.1 (87-112), R= 79.2±3.1 (76-85) Rst= 15.2±1 (14-17), Roes= 24.6±2.26 (21-29), Rv= 8.5±0.75 (8-10), Ran= 5±0.7 (4-6), Rvan= 3.5±0.46 (3-4), Tail= 13.6±3.2 (7-16), VL/VB= ±1±0.16 (0.8-1.4).

Body annuli retrorse, smooth to rough. First annulus of head small, narrow, irregular in outline, not retrorse. Second one retrorse, not set off from succeeding annules. Labial plate absent and submedian lobes small. Vulva open, its anterior flap forms two distinct points in outline. Vagina straight. Tail bluntly rounded. Males were not observed in this study. The species were identified and collected in city Ramsar (Figure 1).

Paratylenchus bukowinensis, Micoletzky, 1922

Measurements Female: (n= 7), L= 348±18.7 (315-372), a= 23.9±8.1 (23.1-27.4), b= 4.1±0.22 (3.7-4.4), c= 9.94±0.85 (8.9-11.1), c‘= 3.1±0.4 (3-3.9), V= 81.6±0.98 (80-83.5), Stylet= 26±1.2 (25.6-28.4), Oeso.= 86.8±3.1 (82-91), S. E. pore= 74.4±4.4 (68-78), H-V= 284.2±15.5 (281-301.8), V= 28.6±1 (26.5-34.4), B.W.= 14.6±1 (14-16), A. B. W= 0.29±0.29 (10-10.7), Tail= 35.3±3.9 (30-39).

Measurements Male: (n= 3), L= 314±15 (311-317), a= 24.1±0.5 (24-25), Stylet= 14±1.1 (11-16), Spicule= 20.2±1.3 (20-20.5), Guber.= 6.4±0.5 (6.2-6.6).

Female: Body bent ventral. Lateral field with four lines. Head rounded with distinct annulations. Rounded submedian lobes protrude very slightly. Spermatheca large rounded, filled with spermatooza. Vulvar flaps small and inconspicuous. Tail evenly conoid, curved slightly ventrad, ending in a rounded to finelrounded terminus.

Males: Stylet very weak, 12-18μm, Spicule 21-25μm long, all juveniles stages with stylet. Pharynx much reduced, non-functional.

Diagnosis: This species is most closely related to P. Lepidus Raski, 1975 from which it differs in its slender, conoid tail shape and large vulgar flaps in P. lepidus (Raski, 1957). The species were identified and collected in city Ramsar (Figure 3).

Paratylenchus elachistus, Steiner, 1949

Measurements Female: (n= 7), L= 342.7±19.5 (314-374), a= 25.7±1.5 (24.3-28.7), b= 4.0±0.13 (3.9-4.2), c= 14.4±2.5 (14.2-17.4), c‘= 2.9±0.49 (2.2-3.7), V= 81.9±0.93 (80.6-83.4), Stylet= 23±0.52 (22-26.6), Oeso.= 84.1±5.4 (76.3-89), S. E. pore= 72.3±5.8 (61.3-77.9), H-V= 280.7±14.7 (258-301), V= 37.4±2.5 (39.4-42), B.W. = 13.3±0.39 (12.5-13.6), A. B. W= 8.2±1.1 (6.8-9.9), Tail= 24.2±6.4 (20-37).

Measurements Male: (n= 1), L= 302, spicule= 12.
Female: body bent ventrad, more in posterior part. Lateral field with four lines. Head conoid to rounded, anteriorly flattened to wide dome-shaped, no protruding lips, stylet delicate. Spermatica with sperm, vulva flaps conspicuous and rounded. Tail thin, terminus spicate or minutely rounded.

Male: Body are almost right and smaller than female, without stylet, pharynx much reduced and non-functional. Length of Spicule 12µm.

This species were identified using identification keys (Brzeski, 1998). This population comes most close to P. longicaudatus Raski,1975 from which it differs by its less anteriorly located vulva (average V= 78 vs. 82-83) and shorter conoid tail. P. elachistus is also related to P. obtusicaudatus Raski,1975 from which it differs by its truncate tail shape and prominently rounded dorso-lateral and ventro-lateral lips which are present in P. obtusicaudatus and arenot present in P.elachistus (Raski, 1957).

Species of the P .lepidus desired species is very similar, but differ in length stylet (27-22 vs 25-19). The species were identified and collected in city Rudsar (Figure. 3).

References


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