

Reported some species of plant parasitic nematodes from rhizosphere of peanut (*Arachis hypogaea*) fields

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Abstract

In order to identify of peanut fields plants parasitic nematodes, 130 samples of soil around the roots of peanut plants were collected in province of Guilan, 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 20 species belonging 17 genera were identified, that are as follows: 1- *Aphelenchoides sacchari* 2-*Aphelenchus avenae* 3- *Basiria graminophila* 4-*Coslenchus costatus* 5-*Ditylenchus myceliophagus* 6-*Filenchus vulgaris* 7-*Helicotylenchus digonichus* 8-*Heterodera cruciferae* 9-*Meloidogyne incognita* 10-*Meloidogyne hapla* 11-*Merlinius bavaricus* 12- *Mesocriconemarusticum* 13- *Mesocriconema curvatum* 14-*Paratylenchus nanus* 15-*Pratylenchus neglectus* 16- *Psilenchus hilarulus* 17-*Quinsulcius capitatus* 18-*Tylenchorhynchus annulatus* 19- *Tylenchorhynchus mashhoodi* 20- *Tylenchus davainei*. In this study, 20 species belonging 17 genera were identified that before just 6 Species 2-5-7-11-15-16 were reported from rhizosphere of Peanut in Iran. Other species (14 Species) are going to report from rhizosphere of Peanut in Iran as a first.

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Introduction

Arachis hypogaea, the peanut or groundnut, is an annual herbaceous plant in the Fabaceae (legume or bean family), with high protein content, vitamins and useful mineral compounds. Total commercial production of peanuts worldwide was 37.6 million tons, harvested from 24.1 million hectares, in 2010. Iran cultivated area The most under cultivating area is located in Guilan province of Iran as 90 percent of peanut crop were cultivated in this area (Anon,2012).

Peanut *Arachis hypogaea* is considered one of the important food crops, produced in many subtropical and tropical countries; also it is a high value cash crop for small and large growers alike. It was listed as one of the twenty crop plants that stand between man and starvation (Wittwer, 1981). Numerous nematode species can damage peanut.(Minton & Baujard, 1990). Several plant parasitic nematodes were reported from peanut fields in South Eastern States of USA such as *Belolaimus longicaudatus*, *Meloidogyne hapla*, *Meloidogyne arenaria*, *Mesocriconema ornatum* and *Pratylenchus brachyurus* (Barker, 1992). Peanut root-knot nematode *Meloidogyne arenaria* is considered one of the most serious nematode pathogens of peanut in many parts of the world, attacking peanut roots, pegs and pods and causing substantial yield losses in severely infested fields (Minton & Baujard, 1990). *Meloidogyne arenaria* and *M. hapla* are the most important peanut crop loss agent in USA, which their populations have reached as economically damaging threshold (Hirunsalee *et al.*,1995). Elekcioglu *et al.* (1994) also have reported many species *Aphelenchus avenae*, *Ditylenchus myceliophagus*, *D. valveus*, *Pratylenchus thornei*, *Tylenchorhynchus goffartri* from peanut fields in the east mediterranean region of Turkey.

In order to improve cultivating steps in peanut and prevent the decline of peanut crop production in traditional cultures, the need to identify plant pathogens, is the first and most important step in

disease management. Since the yield loss associated with plant pathogens including plant parasitic nematodes, this study aims to investigate the peanut nematodes.

Materiales and Methodes

During july to October 2011, 130 soil complex samples were collected from all peanut fields in Guilan province 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. Samples for nematode analysis were stored in a refrigerator until nematode extraction. Nematodes were extracted from soil using De Grisse, 1969 and from root by Coolen & D'Herred techniques (1972). Then extracted nematodes fixed and transferred to glycerin (De Grisse, 1969). In order to identify *Heterodera* spp and *Meloidogyne* spp. Some cuts provided from distal body of female or cyst nematode. 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, 20 species of 17genera of nematodes infraorder Tylenchomorpha were identified as follow: 1- *Aphelenchoides sacchari* 2-*Aphelenchus avenae*3- *Basiria graminophila* 4- *Coslenchus costatus* 5-*Ditylenchus myceliophagus* 6- *Filenchus vulgaris* 7-*Helicotylenchus digonichus* 8-*Heterodera cruciferae* 9- *Meloidogyne incognita* 10-*Meloidogyne hapla* 11- *Merlinius bavaricus* 12- *Mesocriconemarusticum*13- *Mesocriconema curvatum* 14-*Paratylenchus nanus* 15- *Pratylenchus neglectus* 16- *Psilenchus hilarulus* 17-*Quinsulcius capitatus* 18-*Tylenchorhynchus annulatus* 19- *Tylenchorhynchus mashhoodi* 20- *Tylenchus davainei*

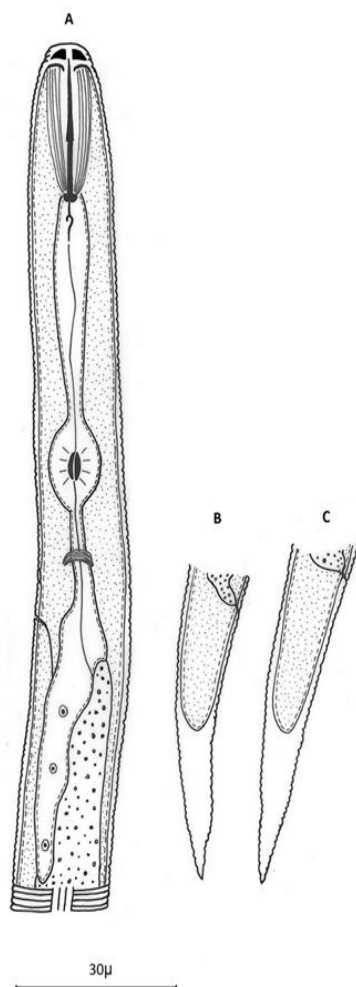
All of the above species previously have been reported from other hosts for parasitic nematodes in Iran, are not new. Six species 2, 5, 7, 8, 15 and 16 have already been reported from the rhizosphere of peanut. Other species (14Species) are going to report for the first time on peanut rhizosphere and are new in Iran. As *Aphelenchoides sacchari*, *Basiria graminophila*, *Tylenchus davainei*, *Coslenchus costatus* and *Filenchus vulgaris* are the common species, have not described in this article. The measurements and descriptions of most of species and some drawing of them are given as following:

1-Heterodera cruciferae Franklin, 1945

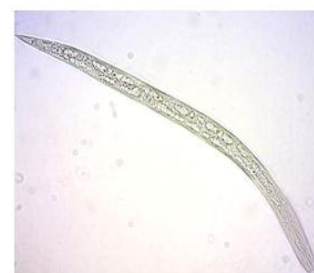
Measurements: J2 (n=9), L=413.3(390-450), a=20.8(18.57-22.9), b= 2.5(2.4-2.76),

b' = 3.6(3.4 -3.7), c= 8.77(7.1-9.88), c' = 3.9(3.36-4.8), Stylet=24(22.4-25), Oeso=159.7(143-172), BW=19.2(17-21), Tail= 46.9(40-57), Hyalin=23.9(20-30.5), H/Stylet=0.99(0.89-1.22), L= 443.4(356-484), W=353(295-445), L/W=1.27(1.1-1.42), Fenestra Width= 46.2(43.5-51), Semi fenestra Length=22(20-25), Vulval slit=47.4(43-51).

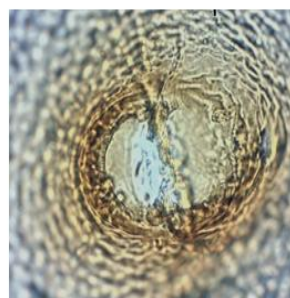
Female: Body swollen, plump, almost spherical, lemon shape with projecting neck containing the oesophagus and part of the oesophagus glands median oesophagus bulb large, sub-spherical with prominent valve. Females are white throughout development, turning brown at death.



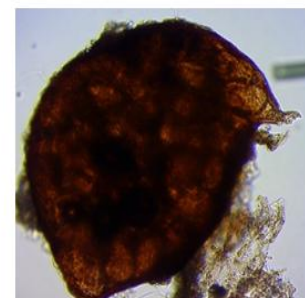
Tail of second stage juvenile



The second stage juvenile of *Heteroderae cruciferae*



Vulva fenestral of Heteroderae



Cyst of *Heteroderae cruciferae*

Fig. 1. The second stage juvenile of *Heterodera cruciferae* A: Anterior body and pharyngeal region B: Variation in tail shape.

Cyst: Broad, almost spherical to lemon shape. Cyst wall tough and dark brown, bearing irregular punctuations, a reticulate pattern of ridges and a subcrystalline layer, the latter lost in old cysts. Cyst is ambifenestrate with very low semifenestral arches separated by a narrow vulval bridge.

In mature cysts, the semifenestrae are unobstructed, but in newly formed cysts, the body wall may still be intact. A narrow under bridge, formed from lateral bands of supporting tissue, connects the proximal end of the vagina with the vulval cone wall; the underbridge may be lost from older cysts. Males were not observed in this study.

Second-stage juvenile: Vermiform, folded three times within egg. Head offset with 3-4 head annules. Esophageal gland extend posteriorly to about 33% of body length. Stylet robust, cone about 40% of total length, basal knobs massive with anterior face flat to concave.

Tail tapering uniformly to a finely rounded terminus, with terminal hyaline zone about 50% tail length and equal to stylet length. Hyaline portion of tail often seen to contain refractive inclusions.

The species were identified and collect in city Astanehashrafiyeh.(Fig.1 and 6)

2-Meloidogyne hapla Chitwood, 1949

Measurements: J2(n= 6), L= 428 (420-435), a= 35.9 (34.1-36.1), b= 4.5 (4.1-5.1) ,b´= 3.4 (3.2-3.6), c= 7.7 (7.5-7.9), c´=6.3 (6.2-6.8), Stylet= 13.3 (13-13.5), Oeso= 129 (125-133), Over= 31 (31-38), BW= 12 (12-12.5), ABW= 9(8-9),Tail= 56 (55-59), Hyalin=13 (12.5-14).

Female: Body pear-shaped with short neck. Cuticle becoming thicker in posterior half of body, sometimes considerably. Head with two annules behind head-cap. Spear knobs rounded, inconspicuous. Posterior cuticular pattern roughly circular, composed of closely spaced smooth or slightly wavy striae. Excretory pore 14-20 annules behind head, hemizonid just posterior to pore. Dorsal arch low. Lateral fields may be unmarked, may be marked only by slight irregularities in the striae, or dorsal and ventral striae may meet at a slight angle along the fields. Some forking of striae at lateral fields may also occur. In some cases ventral striae may extend laterally on one or both sides to form 'wings' which the dorsal striae meet almost at right angles. Tail with few striae but distinct punctuations forming a stippled area between the anus and tail terminus. Sometimes the stippling may be more diffuse over the inner part of the pattern. The juvenile length ranges from 350-470um, and the stylet is fine with small, rounded knobs.

M. hapla developed on many, mainly dicotyledonous, plants, including economic important food crops and ornamentals. It induces small galls, often with secondary roots, *M. hapla* is in Europe the most common and widely distributed root-knot nematode in agricultural areas and natural habitats (Brzeski,1998).

Males were not observed in this study. using key (Jepson, 1987) and This population in comparison with the description (Handoo, 2005) does not show any differences. The species were identified and collected in city Talesh. (Fig.2).

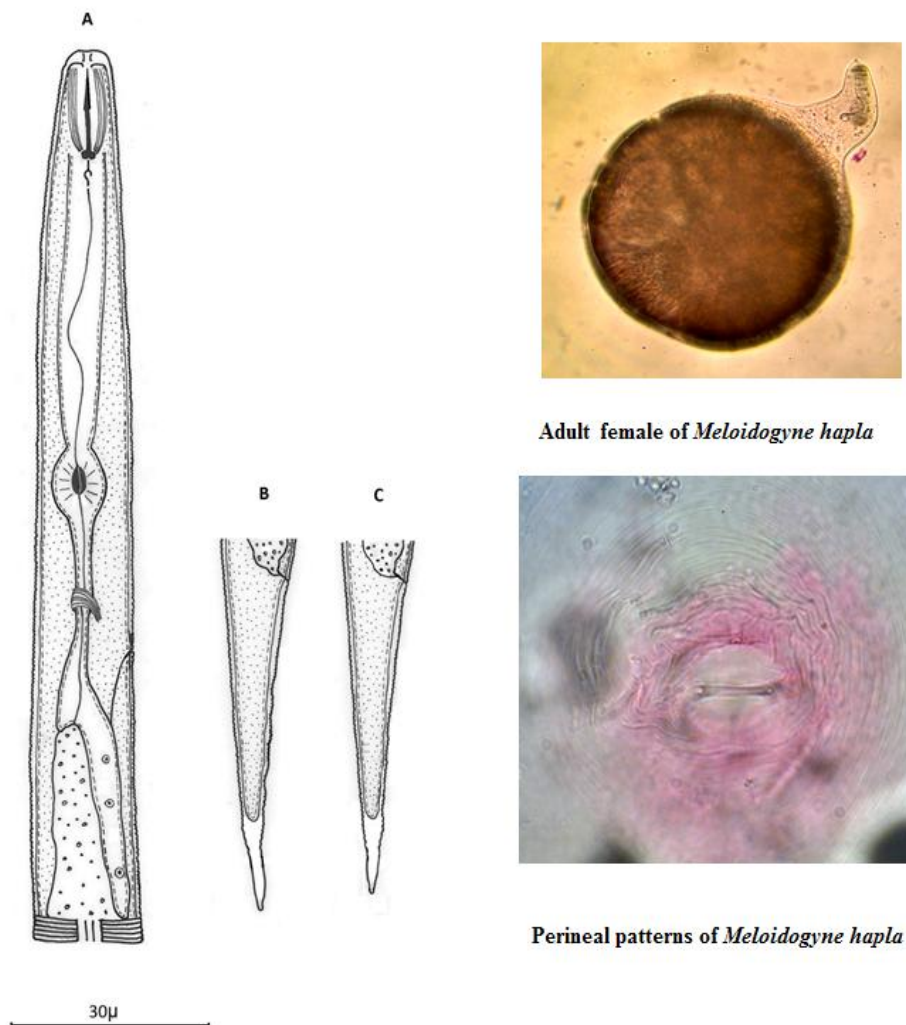


Fig. 2. The second stage juvenile of *Meloidogyne hapla* A: Anterior body and pharyngeal region B: Variation in tail shape.

3-Meloidogyne incognita (Kofoid & White, 1919)
Chitwood, 1949

Measurements female: (n= 7), L=430.5 (412-445), a=35.9 (34.3-37.1), b= 5.1 (4.8-5.2), b' = 3.5 (3.4-3.6) , c= 8.1 (7.7-8.5), c' = 6.6(6.4-7), Stylet=12 (11.5-12.5), Osoe= 127.7 (122-132), Over=38 (36-39), B.W= 12.9 (12-14), A.B.W= 9.1 (8-10), Tail=53 (51-56) ,Hyalin=10.7 (10-11.5).

Female saccate to globose, usually embedded in root tissue which is often swollen or galled; body soft, the neck protrudes anteriorly and the excretory pore is anterior to the median bulb often near the stylet base; the vulva and anus are terminal, flush with or slightly raised from the

body contour; the cuticle of the terminal region forms a characteristic pattern, the perineal pattern, which is made up of the stunted tail terminus, phasmids, lateral lines, vulva and anus surrounded by cuticular striae; the pattern is often characteristic for individual species.

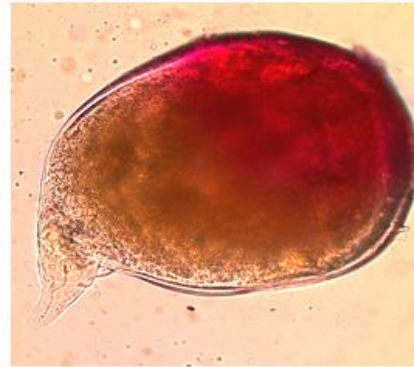
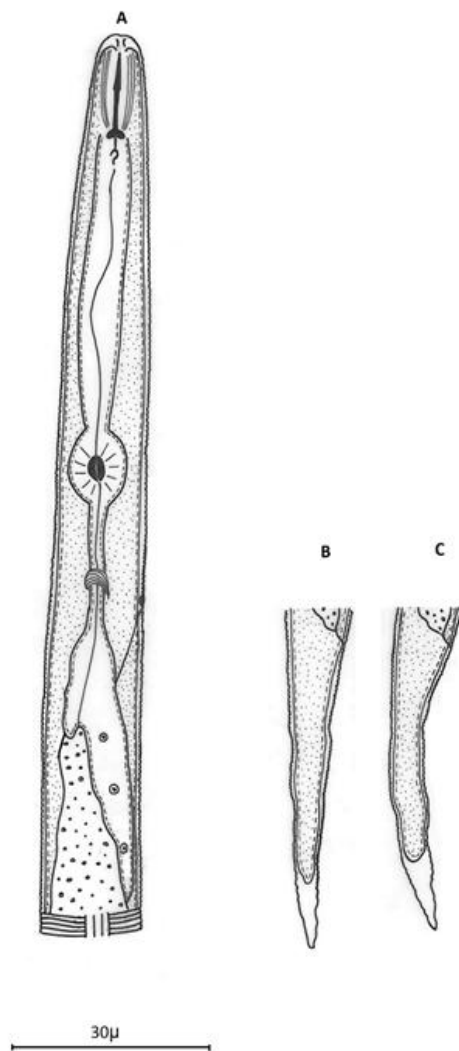
The median esophageal bulb is well developed and the esophageal glands are extensive, overlapping the intestine for several body widths mainly ventrally; the tail is conoid often ending in a narrow rounded terminus but tail length variable, 1.5-7 anal body widths, between species, it often ends in a clear hyaline region the extent of which can help to distinguish species.

Infective second stage juveniles, often free in the

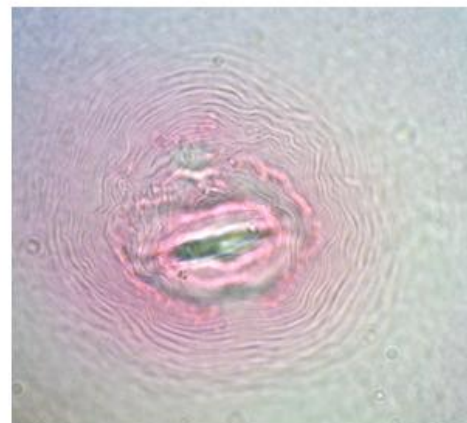
soil, are usually 0.3-9.5 mm long; they are less robust than *Heterodera* juveniles, the stylet is delicate with small basal knobs, under 20 μ m long, and the head skeleton weak.

Males were not observed in this study. Using key (Jepson, 1987) and this population in comparison

with the description (Handoo, 2005) does not show any differences. Males were not observed in this study. The species were identified and collected in city Talesh. (Fig. 3)



Adult female of *Meloidogyne incognita*



Perineal patterns of *Meloidogyne incognita*

Fig. 3. The second stage juvenile of *Meloidogyne incognita* A: Anterior body and pharyngeal region B: Variation in tail shape.

4- *Mesocriconema curvatum* Raski, 1952

Measurements female: (n= 8), L= 411.2 (305-484), a= 10.8 (9.8-12.1), b= 3.8 (3.1-4.2), c= 28.6 (25.4-31.9), c'= 0/7 (0.7-0/9), V= 93.9 (92.7-94.9), Stylet= 54.1 (52-57), m= 78.55 (76.8-79.6), Oeso= 107.5 (100-120), R= 87.13 (80-93), Rst= 13.25 (12-15), Roes= 24.63 (23-

26), Rv= 6.88 (6-7), Ran= 4.88 (4-5), Rvan= 2, Tail= 14.4 (12-17), VL/VB= 1.07 (1-1.3)

Female: Body stout, slightly curved ventrally, tapering slightly toward both extremities. Annules with smooth posterior margins and generally without anastomoses. Head not offset. submedian separate lobes with rounded anterior

margins. First annule broken up into labial plates, often irregularly; second annule transverse; third and following annules retrorse. Vulva open Vagina not sigmoid and straight. Tail conoid-rounded, terminal annule often only faintly indented. Juveniles have irregular margins of annuli when

they are young, margins of larger juveniles smooth (Geraert, 2010). Males were not observed in this study. The species were identified and collect in cities Astaneashrafiye and Kiashahr. (Fig. 4).

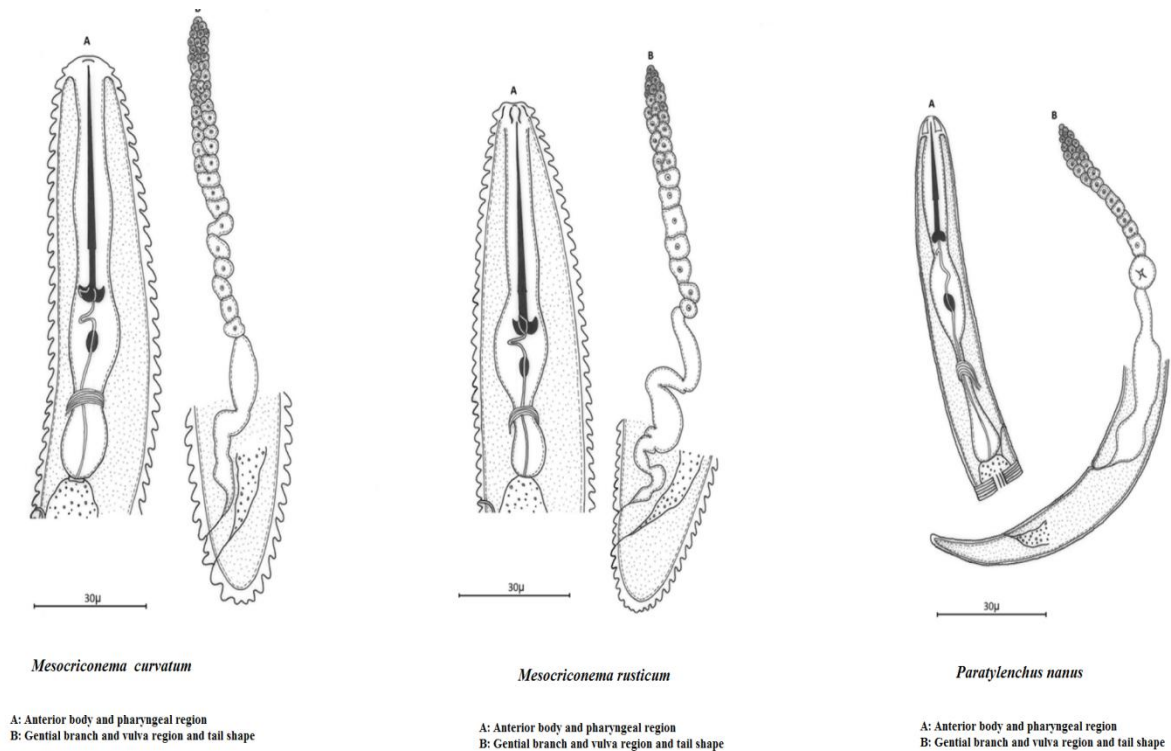


Fig. 4. *Mesocriconema curvatum*, *Mesocriconema rusticum*, *Paratylenchus nanus*.

5- *Mesocriconema rusticum* (Micoletzky, 1915)
Loof & De Grisse, 1989

Measurements female: (n= 10), L= 339.6 (289-382), a= 10.5 (9.2-11.6), b= 3.8 (3.4-4.3), c= 18.2 (15.3-22.6), c' = 0.92 (0.71-1.2), V= 91.66 (89.3-93.3), Stylet= 50.8 (49-54), m= 80.32 (75.5-84.3), Oeso= 89.6 (78-99), R= 106.8 (102-113), Rst= 18.2 (15-18), Rex= 27.6 (25-28), Roes= 27.6 (25-30), Rv= 9.4 (7-12), Ran= 7.4 (5-9), Rvan=1(1-3), Tail= 19.2 (15-25), VL/VB= 1.2 (0.92-1.5).

Female: Body strongly curved ventrally, C-shaped, generally less than 0.5 mm long. Posterior margin of annules smooth. Anastomoses few or none. Head truncate with large submedian lobes. Lip region continuous with contour of body. Spermatheca empty. Vagina straight. Stylet base

anchor-shaped Tail tip curved dorsal, last tail annules generally not extended, giving it a blunt appearance.

Mesocriconema rusticum can be recognized by the open vulva in combination with the large submedian lobes, giving the head a flat appearance, and the blunt, dorsally curved tail (Wouts, 2006).

Males were not observed in this study. The species were identified and collect in cities Astaneashrafiye and Kiashahr. (Fig. 4).

6- *Paratylenchus nanus* Cobb, 1923

Measurements female: (n= 10), L= 311 (290-330), L' = 291 (272-310), a= 23.1 (22.1-24.1),

b= 3.8 (3.3-4.1), c= 15.6 (14.4-16.5), c' = 2.4 (2-2.6), V= 83.8 (80.5-87.8), V' = 89.8 (86.3-93.5), Stylet= 26.5 (24-28), m= 64.5 (63.4-66.6), MB= 56.1 (54.8-58.5), Oeso= 81 (77-86), E-pore= 73.2 (68-77), H-V= 261 (243-290), V-a = 28 (22-38), B.W= 13.4 (13-13.7), A.B.W= 8.2 (7.5-9), Tail= 25.1 (23.5-26)

Female: Body an open 'C' shape after fixation. Head not set off, slightly rounded, in some specimens almost straight sloping sides to rounded anterior end. Submedian lobes very small and close to oral aperture. Lateral lips protrude slightly to give a rounded outline, not truncate. Four or five distinct annules on head. Stylet with posteriorly directed knobs. Ovary outstretched, spermatheca rounded to ovate, filled with spermatozoa only in some of the specimens. Body annules distinct almost to subacute terminus. Lateral field with four incisures equally distinct (Raski, 1957).

P. nanus is closely related to *P. projectus* Jenkins, 1956 and the characters most important in distinguishing these two species are 1- the head region which is truncate, often set off by slight but distinct narrowing, annules indistinct in *P. projectus*, head rounded with distinct annules, not set off, in *P. nanus*; 2- vulva more posterior in *P. projectus* 3- tail bluntly rounded, often digitate in *P. projectus*; subacute in *nanus*; 4- spermatheca lacking or poorly developed in *P. projectus* as contrasted with *P. nanus* with distinct spermatheca sometimes filled with spermatozoa. (Raski, 1957)

Males were not observed in this study. The species were identified and collected in city Rudbar. (Fig. 4).

7- *Quinisulcius capitatus* (Allen, 1955) Siddiqi, 1971

Measurements female: (n= 17), L= 686 (614-844), a= 31.9 (25.1-38.5), b= 4.9 (4.4-6), c= 15.7 (13.1-17.5), c' = 3 (2.5-4.1) V= 56.6 (54.7-58.7), Stylet= 18.4 (17-20), MB= 50.7 (45.5-53.8), , Oeso= 138.6 (112-153), E-pore= 109.8 (103-121), H-V= 388. A.B.W= 14.8 (14-16), Tail= 43.8 (40-53), Annul= 40.4 (35-44) 3 (343-462), V-a= 243.3 (224-263), B.W= 21.5 (19-27),

Female: Body small-sized strongly curving when relaxed. Annules prominent. Lateral fields each with five incisures, smooth or partially areolated. Stylet moderately strong, conus appearing solid anteriorly; knobs rounded, rarely cupped anteriorly. Cephalic region offset, rounded, finely annulated; labial disc indistinct; framework with light to moderate sclerotization. Median bulb well developed, oval, not offset from precorpus by a constriction. Basal bulb large, usually offset from intestine. Cardia prominent. Vulva at 51-59%; lips not modified. Ovaries paired, outstretched. Spermathecae round, axial or slightly offset, rarely functional. Female tail conoid, usually ventrally arcuate, terminal annule enlarged, smooth or striated. Deirids absent. Males rare or absent.

This population comes close to *Q. acutoides* (Thorne & Malek, 1968) Siddiqi, 1971 and *Q. domesticus* Sultan, Singh & Sakhuja, 1995. On the base of their original descriptions they can be separated easily. Species *Q. acutoides* and *Q. domesticus* differs by having smaller stylet and having less annuli in the cephalic region and having less annuli at tail.

In study Males were not observed in this study. The species were identified and collect in cities Astaneashrafiye and Kiashahr (Fig. 5).

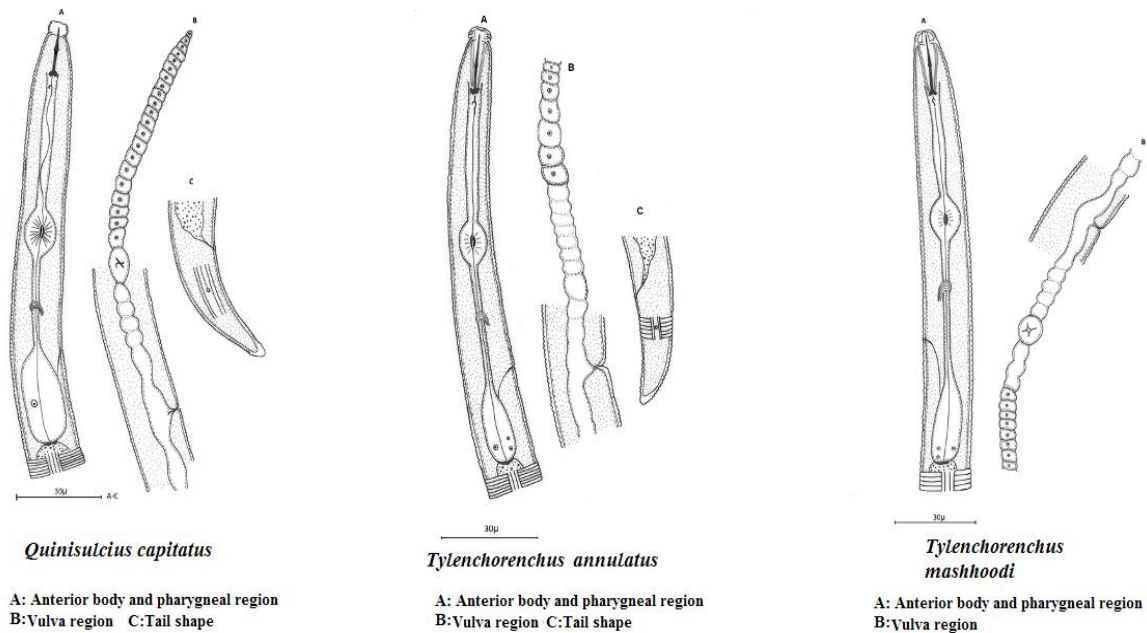


Fig. 5. *Quinisolcius capitatus*, *Tylenchorenchus annulatus*, *Tylenchorenchus mashhoodi*.

8- *Tylenchorhynchus annulatus* (Cassidy, 1930)
Golden, 1971

Measurements female: (n= 10), L= 641 (577-707), a= 37.8 (34.3-41.56), b= 5 (4.4-5.6), c= 14.6 (12.5-16.1), c' = 3.7 (3-5), V= 56.8 (55.4-58.3), Stylet= 17.2 (17-18.5), MB= 46.7 (43.6-49.3), Oeso= 128.6 (110-140), e-pore= 103 (96-108), H-V= 364 (328-398), V-a=248 (242-254), B.W= 17.3 (16-19.2), A.B.W= 12 (10-13.6), Tail=44 (41-47)

Female: Body ventrally arcuate; cuticle moderately thick. Lateral fields with 4 incisures making 3 almost equally wide longitudinal bands; outer incisures crenate; outer bands irregularly areolated in places. Stylet is two almost equal parts, anterior conical part solid, needle-like in distal half, posterior part with distinct, rounded basal knobs. Orifice of dorsal esophageal gland 1.5-2 µm from stylet base, rather obscure. Ovaries paired, outstretched, opposed, with a single row of oocytes. Tail elongate sub-cylindrical, almost straight to slightly arcuate ventrally, with 18-22 annules ventrally and a broadly round, unstriated terminus. Phasmids distinct. Vulva transverse; vagina straight,

extending half-way into body. Spermatheca absent.

This species were identified using identification keys (Brzeski & Dolinski, 1998) and (Handoo, 2000). This population comes close to *T. bohrrensis* Gupta & Uma, 1980 and *T. badliensis* Saha & Khan, 1982. In comparing it with *T. bohrrensis* it has separated from shape of tail and head. *T. annulatus* has offset head and knobs of stylet are subventral.

Males were not observed in this study. The species were identified and collected in city Rudbar. (Fig.5).

9- *Tylenchorhynchus mashhoodi* Siddiqi & Basir, 1959

Measurements female: (n= 7), L= 675.1 (623-730), a=31.8 (29.7-35), b= 4.6 (4.3-4.8), c= 14.4 (13.6-15.5), c' = 3.1 (2.9-3.3), V= 55.8 (54.8-56.6), Stylet= 19.7 (18.6-20), Lip Annuli= 3, MB= 46.3 (44.6-48), Oeso= 147 (140-156), H-V= 376.5 (353-403), H-a= 253 (250-256), B.W= 21.3 (19-23), A.B.W= 15.1 (14-16), Tail= 47.5 (46-52), Annul= 30 (30-35).

Female: Body slightly ventrally arcuate to varying degrees when relaxed. Head rounded, continuous with body or slightly set off, 6.5µm wide, 3.6µm high, bearing three large annules. Lateral field about 30% of body diameter, outer of four incisures strongly crenate. Excretory pore opposite anterior end of basal esophageal bulb. Stylet knobs round with sloping or concave anterior surfaces. Dorsal gland orifice 2.5µm posterior. Ovaries outstretched, oocytes in single rows; spermatheca spherical. Vagina depth no more than half body diameter. Tail uniform conoid, slightly arcuate, ventral surface with 13-16 irregularly annules. Tail tip large, smooth, bluntly conoid. Phasmids well anterior to midtail.

This population comes close to *T. manubriatus* Litinova, 1946 and *T. kashmirensis*, Mahajan, 1974. It differs from the first by having less annuli on the head, and from the second by having longer stylet (Handoo, 2000). Males were not observed in this study. The species were identified and collected in city Kiashahr (Fig. 5).



Fig. 6. Field symptoms showing patchy distribution of damage caused by groundnut plants infected by *Heterodera crucifera*.

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