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## SEVEN NEW RECORDS OF PLANT PARASITIC NEMATODES FROM DISTRICT BAJAUR, KPK, PAKISTAN

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### ABSTRACT

District Bajaur is a newly merged District of Khyber Pakhtunkhwa Pakistan. The total areas of Bajaur are 129035 hectare. It is located at an elevation of 1152 meters above sea level. The total land under agriculture is 77166 hectare. In Bajaur the cultivated field under irrigation covers approximately 2739.22 hectares and over 74426 hectares is barani (irrigated) and around 20003.99 hectares is uncultivated. Of the total cultivation fields Three fourths depends upon precipitation and managed by farmers. The current study was carried out in twelve different location of district bajaur KPK Pakistan. Four zone were selected from district bajaur on the bases of mountainous areas, plane areas, availability of water and soil fertility; Total 200 soil samples were collected from the study area during August 2018 to September 2020 for diagnosing the nematode problems. Out of 200 samples seven new record species were identified viz., *Aphelenchoides shamimi* Khera 1970; *Filenchus uliginosa* (Brzeski, 1977) Siddiqi, 1986; *Helicotylenchus africanus* (Micoletzky, 1916) Andrassy, 1958; *H. digitiformis* Ivanova, 1967; *Psilenchus curcumerus* Rahaman, Ahmad & Jairajpuri, 1994; *Pratylenchus wescolagricus* Corbett, 1984; *Tylenchorhynchus hordei* Khan1972.

**Keywords:** Agriculture; Plant parasitic nematodes; Taxonomy; Bajaur, KPK.

### INTRODUCTION

Agricultural production is the pillar of Pakistan's economy. Economy of Pakistan depends on the development of agriculture (Ali *et al.*, 2013). Several surveys have been carried out in Pakistan to investigate the genera of nematodes associated with several important crops and the damage caused by nematodes on the crops. Plant parasitic nematodes causing a loss of US\$ 173 billion all over the world (Elling, 2013). Parasitic nematodes are widespread in nature and have almost all important crops associated with agriculture and pose major food safety constraints.

Many surveys have been panned during 2018-2020 to capture and identify the nematode from twelve different location of district bajaur KPK Pakistan. Four zone were selected on the bases of mountainous areas, plane areas,

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availability of water and soil fertility. Soil samples were collected from four different zones of the region. About 200 soil and root samples were collected, the results of these samples have shown prevalence of 22 nematode species belonging to 12 genera, 11 families and 5 orders viz., *Aphelenchida*, *Dorylaimida*, *Enoplida*, *Rhabditida* and *Tylenchida*. The result shows that frequency of occurrence of nematode species encountered during the survey was maximum in the order Tylenchida (45%) followed by the order Rhabditida (25.4%), Dorylaimida (15.4%), order Aphelenchida (10.5%), Mononchida (3.5%) and Plectida (2.2%). From those samples seven new record species were identified *Aphelenchoides shamimi* Khera 1970; *Filenchus uliginosa* (Brzeski, 1977) Siddiqi, 1986; *Helicotylenchus africanus* (Micoletzky, 1916) Andrassy, 1958; *H. digitiformis* Ivanova, 1967; *Psilenchus curcumerus* Rahaman, Ahmad and Jairajpuri, 1994; *Pratylenchus wescolagricus* Corbett, 1984; *Tylenchorhynchus hordei* Khan1972.

### MATERIAL AND METHODS

During the current studies a total of 200 soil and root samples were taken from the soil around the roots of

agrarian site of district bajaur KPK Pakistan. The samples were placed in polythene bags and sealed tightly with a rubber band with relevant information including date of collection, locality, and crop detail was mentioned on each bag. Soil and root samples were processed by the methods of Cobb (1918) and Baermann (1917) funnel method. Nematodes were killed fixed and processed by Courtney *et al.*, (1955), Seinhorst (1959) methods. Identification of nematodes was made through measurements given by De Man (1884) formula with an ocular micrometer under a compound microscope and identification was based on the systematics given by Siddiqi (2000). Illustrations were drawn by drawing tube attached to the Nikon Eclipse E400 microscope. Photomicrographs were also captured compound microscope using Nomarski's interference contrast system (Nikon DS-Fi1)

## RESULTS

*Aphelenchoides shamimi* Khera 1970: (Figure 1, 2)

Measurements: Females (n = 15): L = 500-681 (550 ± 20)µm; a = 29-32 (30 ± 2.4); b = 5.3-6.3 (5.7 ± 0.5); c = 15-20 (18 ± 0.8); V = 70-73.6 (60 ± 5.3)%; Stylet = 8-11 (10 ± 0.8) µm; Pharynx = 77-101 (88 ± 3.4) µm; Tail = 28-35 (32 ± 2.1) µm; Anal body width = 8-12 (10 ± 0.4) µm; Maximum body width = 16-18 (16.5 ± 1.2) µm.

Males (n = 7): L = 500-562 (545 ± 22) µm; a = 30-35 (31 ± 2.1); b = 5.0-6.2 (5.5 ± 0.5); c = 15.7-18.4 (17 ± 0.6); Stylet = 7-8 (7.5 ± 0.3) µm; spicules = 14-18 (16 ± 0.7)µm;

gubernaculum = 8-11 (10 ± 0.4) µm; Pharynx = 83-99 (87 ± 10) µm; Tail = 30-33 (31 ± 2.1) µm; Anal body width = 10-12 (11 ± 0.6) µm; Maximum body width = 16-17 (16.5 ± 0.5) µm.

**Description: Female:** Transparent body posteriorly curved slightly ventral upon relaxation. Cuticle is with fine striation. Five incisures were present in lateral field. Head is set-off from the body, with six lip papillae which is not clearly visible. Head is with sclerotized framework. Stylet is present with a poor basal knob. Pharynx has a slender procarpus and well developed median bulb with a visible crescentic valve plates. Nerve ring is present. Ovary single, long, outstretched extending posterior to the margin of esophageal gland. Oocytes arranged in a single row. Spermatheca is small. Tail is bluntly rounded with terminus having a ventrally situated mucron.

**Male:** The posterior end of the body is curved little more than in female. Head, lip, stylet, cuticle, lateral fields, esophagus, position of nerve ring and excretory pore is like female. Testes is single, spermatocytes are in a single row than in double and then in multiple row finally. Two spicules present of equal sized. Gubernaculum is not present.

**Remarks:** The above mentioned species was collected from the soil around peach (*Prunus persica* L.) Alizo and Mumando closely resembles to *Aphelenchoides shamimi* Khera 1970.

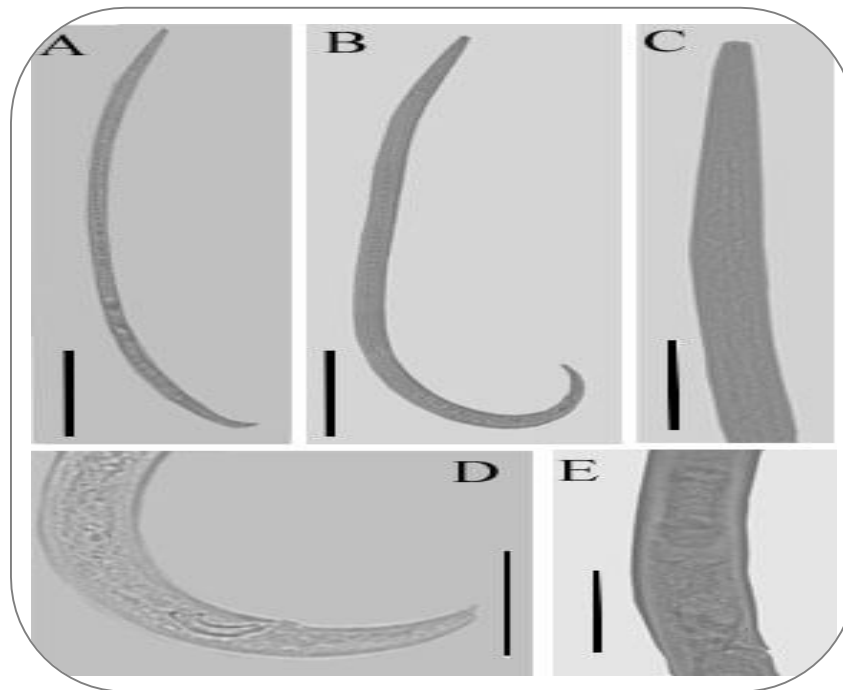


Figure1. Light microphotograph of *Aphelenchoides shamimi* Khera 1970 (A-E). Female A. whole body; C. pharyngeal region; E. vulval region; (male) B. whole body; D. tail region showing spicule.

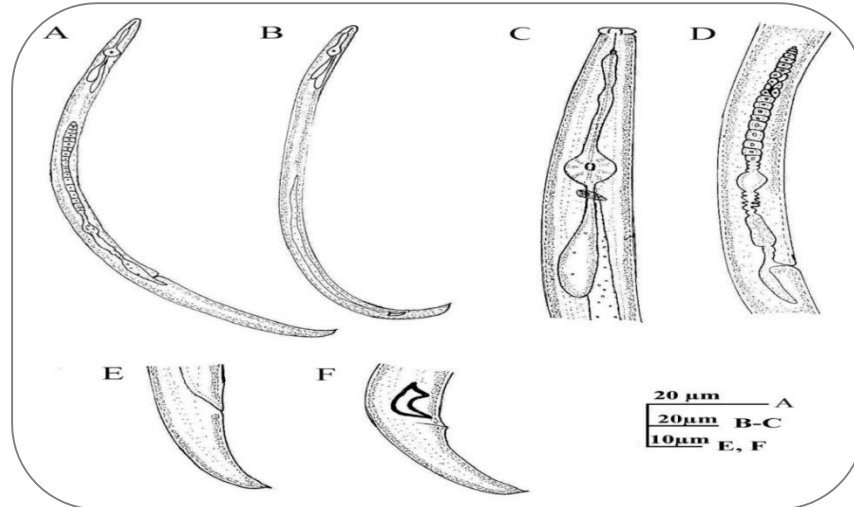


Figure 2. *Aphelenchoides shamimi* Khera 1970 Female (A,C, D, E) A. whole body; C. pharyngeal region; D. vulval region; E. tail region; (male) B. whole body; F. tail region showing spicule.

***Filenchus uliginosa*** (Brzeski, 1977) Siddiqi, 1986: (Figure 3-4)

**Measurements:** Females (n = 5): L = 630-734 (666 ± 25) µm; a = 31-41 (34 ± 3.1); b = 6-6.7 (6.4 ± 0.6); c = 4.8-6.4 (5.0 ± 0.1); V = 57-62.5 (60 ± 2.1); stylet = 10-12 (11 ± 0.3) µm; Pharynx = 105-125 (116 ± 11) µm; Tail = 103-145 (130 ± 10.5) µm; Anal body width = 10-14 (12 ± 0.6) µm; Maximum body width = 16-23 (20 ± 0.5) µm.

Males (n = 7): L = 630-749(713 ± 23)µm; a = 29-40 (34.7 ± 2.9); b = 5.4-6.5 (5.8 ± 0.7); c = 3.6-5.9 (4.8 ± 0.5); stylet = 8-12 (10 ± 0.4) µm; spicules = 18-23 (20 ± 1.2) µm; gubernaculum = 5-8 (10 ± 0.3) µm; Pharynx = 112-130 (120 ± 9) µm; Tail = 130-193 (170 ± 11) µm; Anal body width = 12-15 (13 ± 0.8) µm; Maximum body width = 15-23 (20 ± 0.6) µm.

**Description: Female:** Large nematodes having thick cuticle annulations. Body straight after heat relaxation. Lateral field with four incisures. Labial region is continuous from the body, having 4-5 annules, six lip papillae and four cephalic papillae. Stylet is present with a thick and large basal knob. DGO about 1-2 µm long. Excretory pore situated at the base of isthmus or isthmus and basal bulb junction. Nerve ring present. Pharynx has a slender procarpus and weak median bulb. Ovary single, long, outstretched extending posterior to the margin of esophageal gland. Oocytes arranged in a single row. Spermatheca is filled with sperms. Filliform tail present.

**Male:** Same as female in morphology excepted stylet, bursa, spicules and gubernaculum.

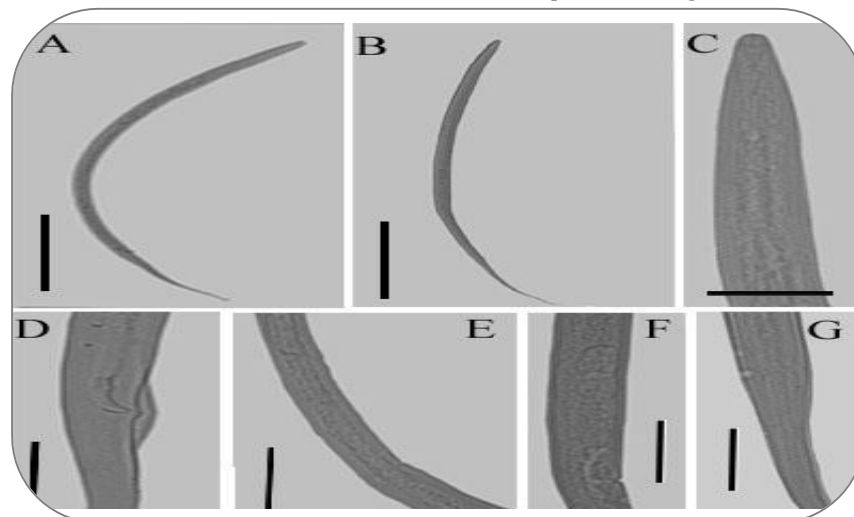


Figure3. *Filenchus uliginosa* (Brzeski, 1977) Siddiqi, 1986. Light microphotograph of (A-G). Female B. whole body; C. pharyngeal region; E, F. vulval region; G. tail region; (male) B. whole body; D. tail region showing spicule and gubernaculum.

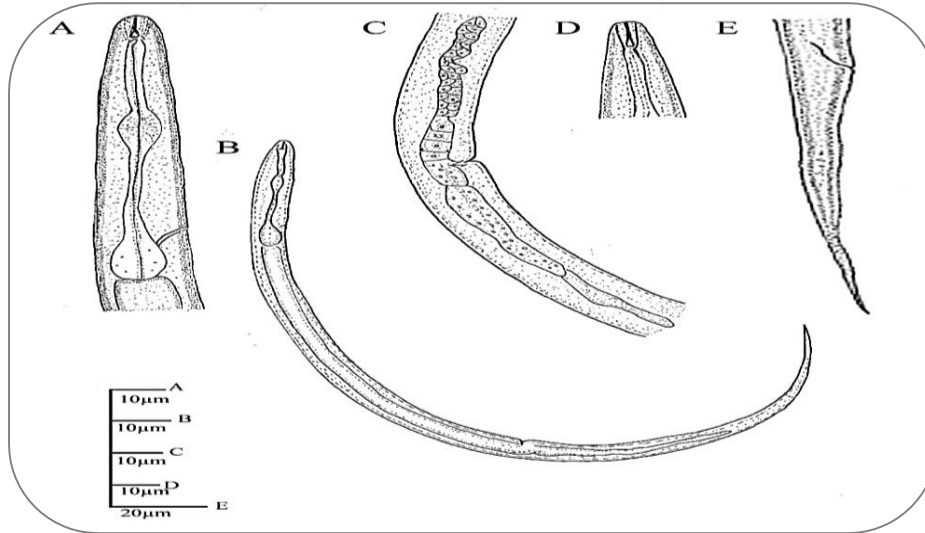


Figure 4. *Filenchus uliginosa* (Brzeski, 1977) Siddiqi, 1986. (A-G). Female A. Pharyngeal region; B. whole body; C. vulval region; D. Anterior region; E. tail region.

**Remarks:** The above mentioned species was collected from the soil around garlic (*Allium sativum* L.) Alizo, Khar and Mumando closely resembles to *Filenchus uliginosus* (Brzeski, 1977) Siddiqi, 1986.

***Helicotylenchus africanus*:** (Micoletzky, 1916) Andrassy, 1958 (Figure5-6)

**Measurements:** Females (n = 5): L = 600-726 (631 ± 32) µm; a = 20-27 (24 ± 2.1); b = 4.8-5.0 (4.8 ± 0.6); c = 27-41 (28 ± 1.6); stylet = 26-29 (28 ± 2.1) µm; Pharynx = 120-160 (140 ± 12) µm; Tail= 15-22 (20 ± 0.9) µm; Anal body width = 12-14 (12 ± 0.7) µm; Maximum body width = 22-26 (24 ± 1.1) µm.

**Males:** Not Found

**Female:** Body C shaped to spiral 600-726 (631±) µm long. Lip region rounded with 4-6 annuli. Spear knob is

2-4µm in length. DGO is present 7-11µm behind spear knob. Median bulb is ovate, 12-16µm long and 7-12 µm wide. Pharynx is 120-160 µm long and excretory pore is 100-120µm from head. Hemizonid is present anterior to excretory pore. Reproductive systems have two branches and both are functional and equally developed. Spermatheca off set and fill with sperm. Phasmid is present anterior to anus. Tail is 15-25µm long and dorsally curved with an annulated round projection, occasionally pointed with 10-18 ventral annuli.

**Remarks:** The above mentioned species was collected from the soil around Loquat (*Eriobotroirya japonica* L.) all localities of bajaur closely resembles to *Helicotylenchus africanus* (Micoletzky, 1916) Andrassy, 1958.

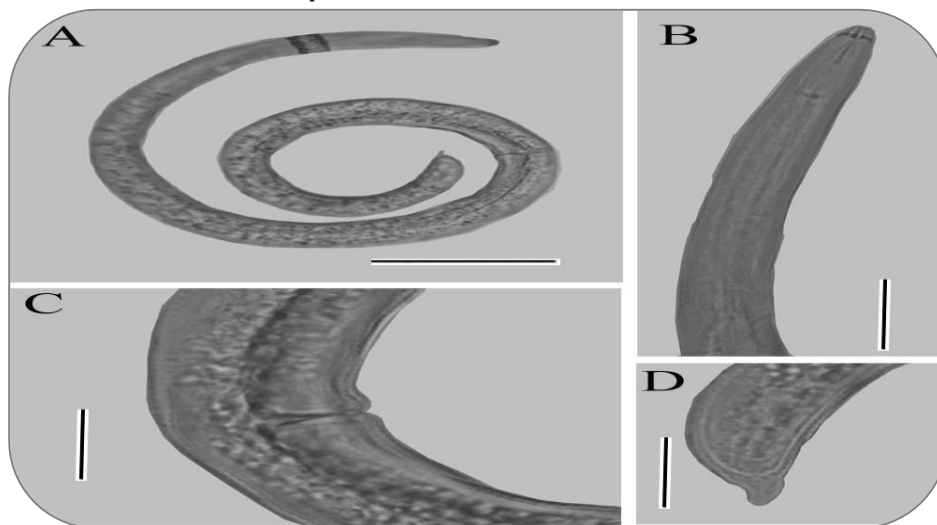


Figure5. Light microphotograph of *Helicotylenchus africanus* (Micoletzky, 1916) Andrassy, 1958 (A-D). Female A. whole body; B. pharyngeal region; C. vulval region; D. tail region.

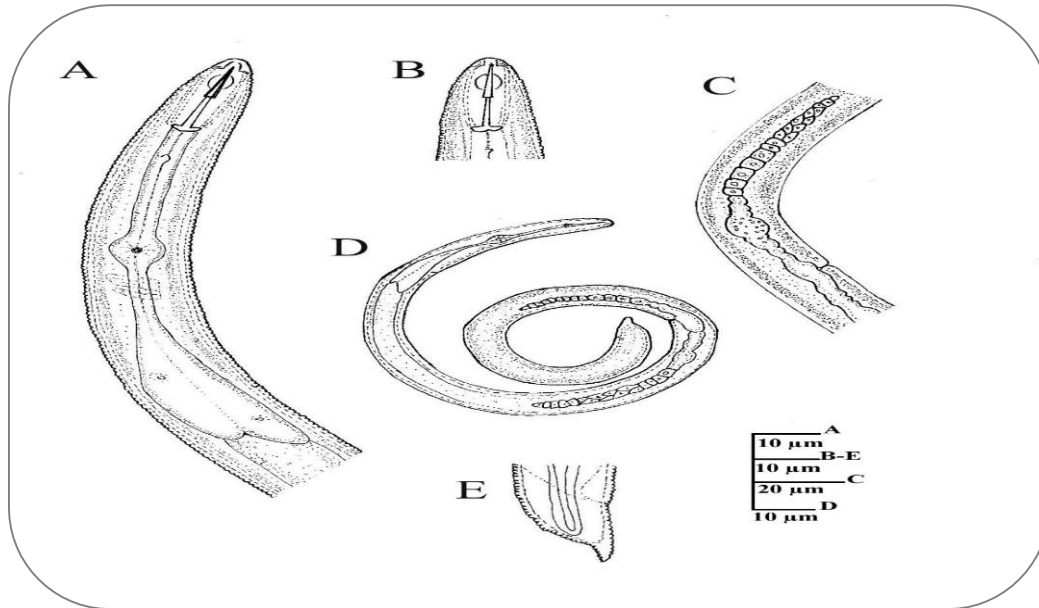


Figure6. *Helicotylenchus africanus* (Micoletzky, 1916) Andrassy, 1958 (A-E) A. pharyngeal region; B. anterior region; C; vulval region; D. whole body; E. tail region.

***Helicotylenchus digitiformis* Ivanova, 1967:** (Figure7-8)

**Measurements:** Females (n = 5): L = 546-650 (609 ± 23)μm; a = 27-29 (28 ± 1.1); b = 4.2-5.4 (4.9 ± 0.7); c = 28-38(30 ± 2.1); V = 61-65 (63 ± 3.4); stylet = 25-29 (27 ± 1.4) μm; . Pharynx = 115-150 (130 ± 3.1) μm; Tail= 16-23 (21 ± 1.3) μm; Anal body width = 12-14 (13 ± 0.7) μm; Maximum body width = 22-24 (23 ± 0.9) μm.

**Males:** Not Found

**Description: Female:** Body c shaped to spirals upon fixation. Cuticular rings are 1.2 μm wide at mid body. Lateral fields with four incisures, occupies one fourth of the body width Lip region is conical, continuous with five cuticular rings. Anteriorly concave spear knob, Excretory pore is

present at 95-115μm from anterior side. Procorpus is tube shaped structure and having well developed and spherical metacarpus. Nerve ring present which encircling narrow isthmus. Ovaries paired and straight, oocytes arranged in single layer and form two layers at mid ovary. Spermatheca is present which is nonfunctional. Tail narrows dorsally with a wide tip and ventrally with a characteristic digitate outgrowth which curved toward dorsal side, 3-4 ring present on digitate outgrowth.

**Remarks:** The above mentioned species was collected from the soil around Loquat (*Eriobotroirya japonica* L.) all localities of bajaur instead of arang and batwar, closely resembles to *Helicotylenchus digitiformis* Ivanova, 1967.

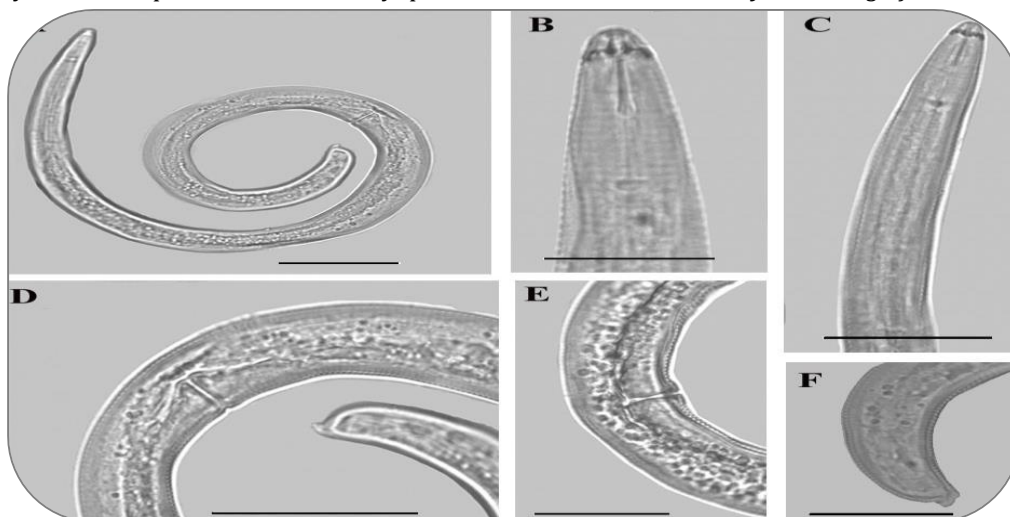


Figure7. Light microphotograph of *Helicotylenchus digitiformis* Ivanova, 1967 (A-E). Female A. whole body; B. anterior region; C. pharyngeal region; D, E. vulval region; E. tail region.

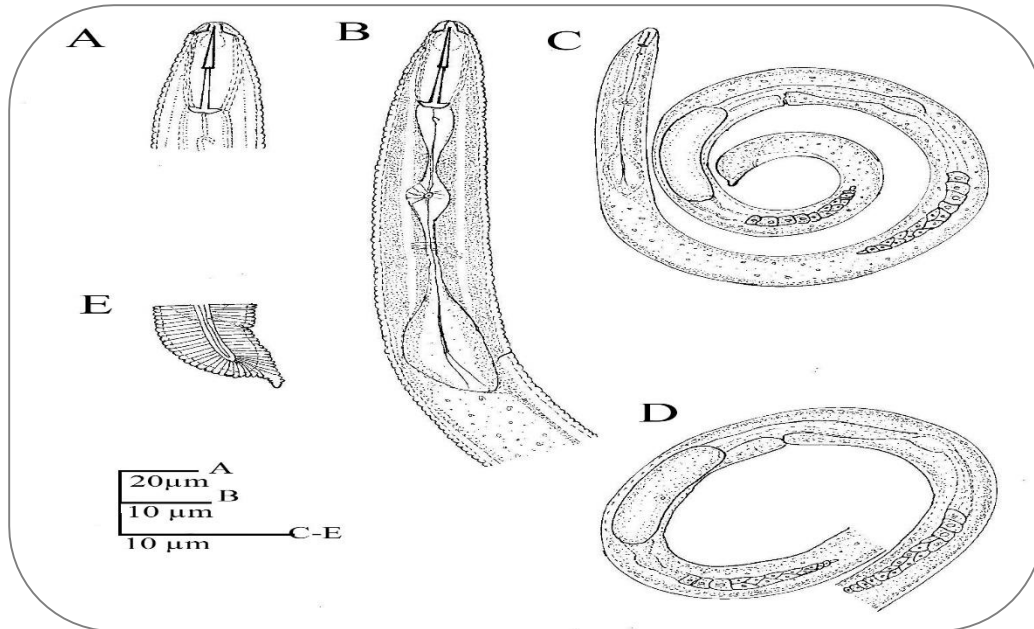


Figure 8. *Helicotylenchus digitiformis* Ivanova, 1967 Female (A-D) A. anterior region; B. pharyngeal region; C; whole body; D. vulval region; E. tail region.

***Pratylenchus wescolagricus* Corbett, 1984:** (Figure 9-10)

Measurements:

**Females** (n = 5): L = 551-688 ( $633 \pm 21$ )  $\mu\text{m}$ ; a = 28-40 ( $35 \pm 1.1$ ); b = 4.2-5.6 ( $4.9 \pm 0.7$ ); c = 16-30 ( $23 \pm 2.1$ ); V = 75-82 ( $78 \pm 4.6$ ); stylet = 10-18 ( $15 \pm 1.4$ )  $\mu\text{m}$ ; . Pharynx = 90-137 ( $130 \pm 3.1$ )  $\mu\text{m}$ ; Tail = 24-35 ( $27 \pm 2.1$ )  $\mu\text{m}$ ; Anal body width = 9-13 ( $10 \pm 0.4$ )  $\mu\text{m}$ ; Maximum body width = 16-20 ( $17 \pm 0.6$ )  $\mu\text{m}$ .

**Description: Female:** straight or curved body ventrally on relaxation. Well-developed annulation, tapered from vulva to tail. Lateral field having four inscers. Two outer layers are areolated irregularly, sometime oblique striation present in mid body on middle band, sometime an extra band showing the appearance of six lines. Rounded labial region with four annuli, 10-15 annuli present on tail. Labial framework is massive having oval oral aperture. Stylet strong with angulate basal knob, 2-4  $\mu\text{m}$  posterior dorsal gland opening is present. Oval median bulb and nerve ring is well developed enclosing narrow isthmus at anterior part of glandular pharynx. Posterior part of pharynx overlapped the intestine laterally and ventrally by 20-50  $\mu\text{m}$ . Excretory pore is present at 80-95  $\mu\text{m}$  from anterior end, slightly anterior to pharyngo- intestinal junction. Hemizonid is present in front of excretory pore. Single anterior ovary is present, small and nonfunctional spermatheca is present.

Cuticle on dorsal body is present just opposite to anus

with double annulation. Tail rounded smooth often indented

**Male:** not found

**Remarks:** The above mentioned species was captured from the soil around Loquat (*Eriobotroirya japonica* L.) major localities of bajaur like Arang, Hajilawang, and Gambat, closely resembles to *Pratylenchus wescolagricus* Corbett, 1983.

***Psilenchus curcumerus*** Rahaman, Ahmad & Jairajpuri, 1994 (Figure 11-12)

**Measurements:** Females (n = 4): L = 854-925 ( $910 \pm 34$ )  $\mu\text{m}$ ; a = 30-42 ( $38 \pm 2.1$ ); b = 6.4-6.7 ( $6.5 \pm 0.3$ ); c = 6.4-6.7 ( $6.5 \pm 0.3$ ); V = 45-47.6 ( $46 \pm 1.1$ ); stylet = 10-16 ( $15 \pm 0.6$ )  $\mu\text{m}$ .

Males (n = 6): L = 795-930 ( $885 \pm 21$ )  $\mu\text{m}$ ; a = 42-52 ( $48 \pm 2.1$ ); b = 5.2-6.8 ( $6.4 \pm 0.5$ ); c = 5.0-5.6 ( $5.3 \pm 0.3$ ); stylet = 10-15 ( $12 \pm 0.7$ )  $\mu\text{m}$ ; spicules = 25-31 ( $28 \pm 1.0$ )  $\mu\text{m}$ ; gubernaculum = 8-10 ( $9 \pm 0.6$ )  $\mu\text{m}$ .

**Female:** Slender body ventrally curved after heat relaxation, tapering anteriorly from base of pharynx, posteriorly from terminating as a filiform tail with clavate terminus. Cuticle having fine striation, each striae is less than 1.0  $\mu\text{m}$  wide at middle body and 2-3  $\mu\text{m}$  at post anal region. Lateral fields with three ridges at mid body starting from base of spear and ending on just behind of anus. The outer ridges areolated from anterior end to tail. Labial region rounded, continuous and smooth. Weakly sclerotized cephalic framework.

Stylet narrow and spear cone is one third of stylet length. Orifice of dorsal oesophageal gland is about 1.0-3µm behind the stylet base. Procorpus is 55-66µm long, muscular metacarpus. Metacarpus is 14-19µm long, located at 55-57% of pharynx length. Isthmus is narrow, 26-35µm long. Basal bulb is oval, 19-22µm long and round. Nerve ring is present 90-104µm from anterior end. Excretory pore is 95-105µm from

anterior. Hemizonid is slightly anterior to excretory pore. Amphidelphic gonads and oocytes are arranged in a single layer at anterior and posteriorly one or two rows. Spermatheca is oblong, 20-32 µm long. Tail elongate, filiform with a clavate terminus.

**Male:** Similar to female but slightly smaller body as of female, having ventrally curved spicule, Gubernaculum is trough shaped and ad anal bursa is present.

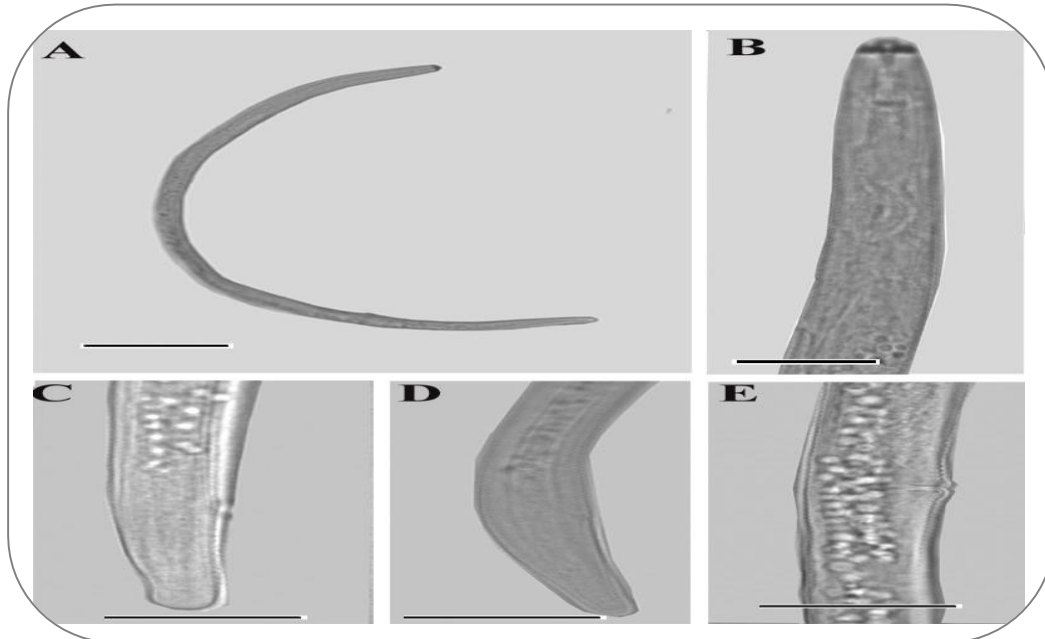


Figure 9. Light microphotograph of *Pratylenchus wescolagricus* Corbett, 1983 (A-E). Female A. whole body; B. pharyngeal region; C,D.; tail region; E. vulval region.

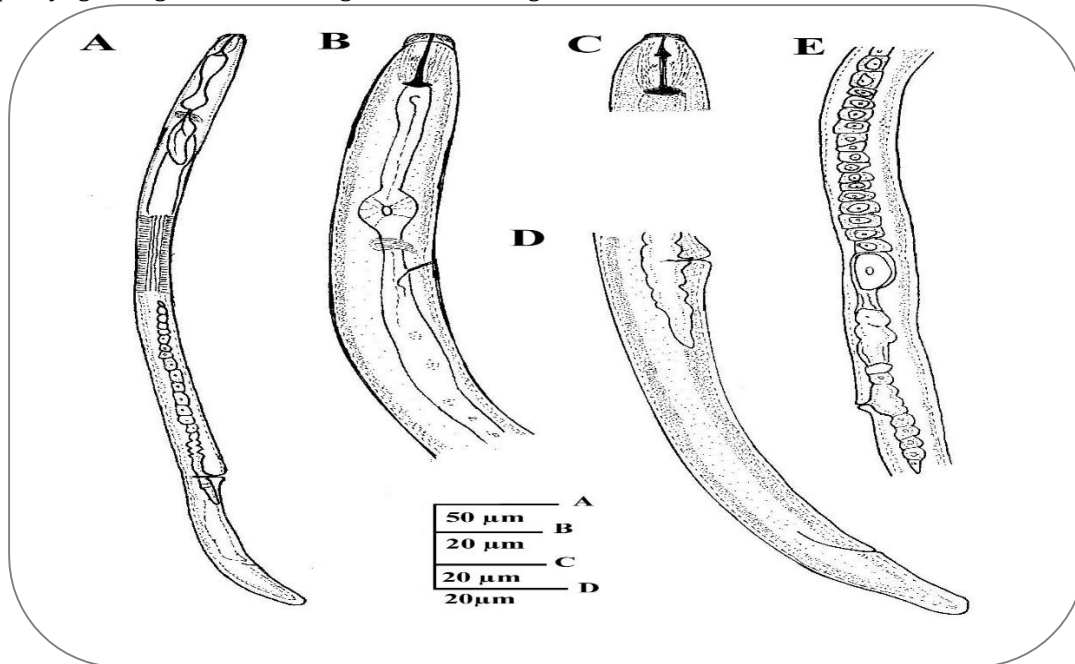


Figure 10. Light microphotograph of *Pratylenchus wescolagricus* Corbett, 1983 (A-F). Female A. whole body; B. pharyngeal region; C, anterior region; D.vulval region with tail; E. vulval region.

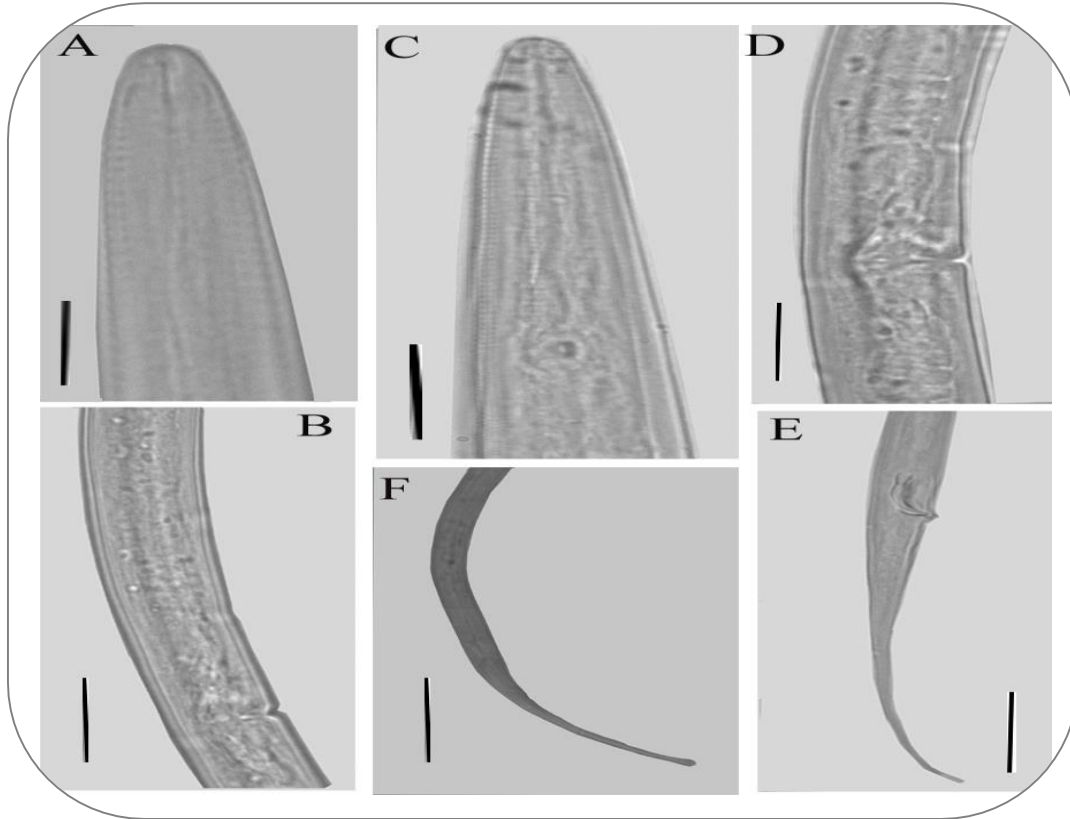


Figure 11. Light microphotograph of *Psilenchus curcumerus* Rahaman, Ahmad & Jairajpuri, 1994 (A-F). Female A. whole body; B. vulval region; C,D. pharyngeal region; F. tail region; (male) E. tail region showing spicule.

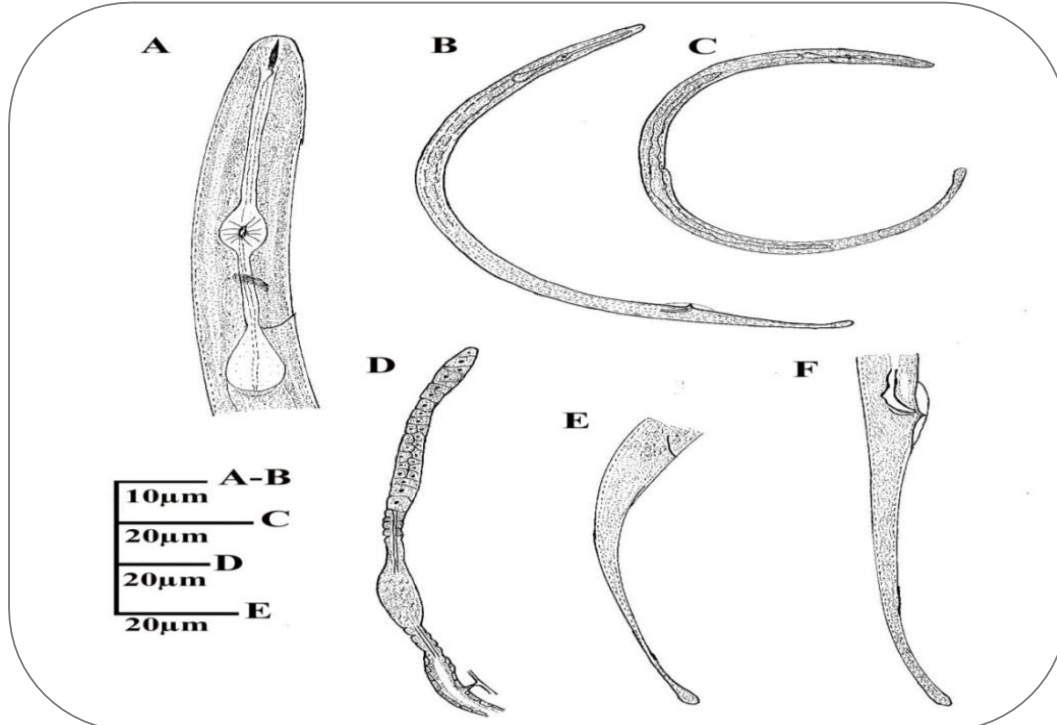


Figure 12. Light microphotograph of *Psilenchus curcumerus* Rahaman, Ahmad & Jairajpuri, 1994 (A-F). Female A. pharyngeal region; C. whole body; D.vulval region; E. tail region; male: B. whole body; F tail showing spicule.



**Remarks:** The above mentioned species was found from the soil around okra (*Abelmoschus esculentus* L.) from Arang, closely resembles to *Psilenchus curcumerus* Rahaman, Ahmad & Jairajpuri, 1994

***Tylenchorhynchus hordi* Khan 1972:** Figure (13-15)

**Measurements:** Females (n = 5): L = 615-641 (621 ± 21)µm; a = 27-30 (29 ± 1.2); b = 4.2-5.0 (4.9 ± 0.3); c = 13.5-14.3(14 ± 0.4); V = 53-62.5 (60 ± 1.3); stylet = 18-19 (18.5 ± 0.5) µm.

Males (n = 7): L = 592-657 (642 ± 20) µm; a = 31.2-35.8 (34.7 ± 1.4); b = 4.6-5.2 (4.8 ± 0.2); c = 13.9-15.7 (14.4 ± 0.4); stylet = 18-19 (18.5 ± 0.9) µm; spicules = 23-27 (25 ± 1.4) µm; gubernaculum = 8-11 (10 ± 0.1) µm.

**Description: Female:** Body slightly arcuate ventrally. Annules averaging 1.0-1.4 µm wide at mid-body. Four incisures present in lateral fields. Head offset from body, lip region rounded, with 4-5 annules; head framework

some time sclerotized, Stylet 18-19 (18.5) µm long; cone of stylet is longer than the shaft; knobs rounded, backwardly directed. Dorsal gland orifice 3.2 µm behind from stylet base. Hemizonid 1-2 body annules long, 2-4 annules anterior to excretory pore, or at the base of isthmus. Pharynx physically as of the genus, basal bulb clearly setoff from intestine. Excretory pore located 87-104 µm from head. Vulva a transverse slit without epiptygma. Ovaries didelphic or amphidelphic 53-62% of female body. Spermatheca rounded, with sperms. Tail cylindrical, 2-3 anal body widths long, bluntly rounded or smooth terminus, 40-46 annules on its ventral side of tail. Phasmids slightly anterior to middle of tail.

**Male:** Similar to female in general morphology. Spicules tylenchoid, cephalate, with pointed distal end. Gubernaculum rod-like, with proximal end directed forward when

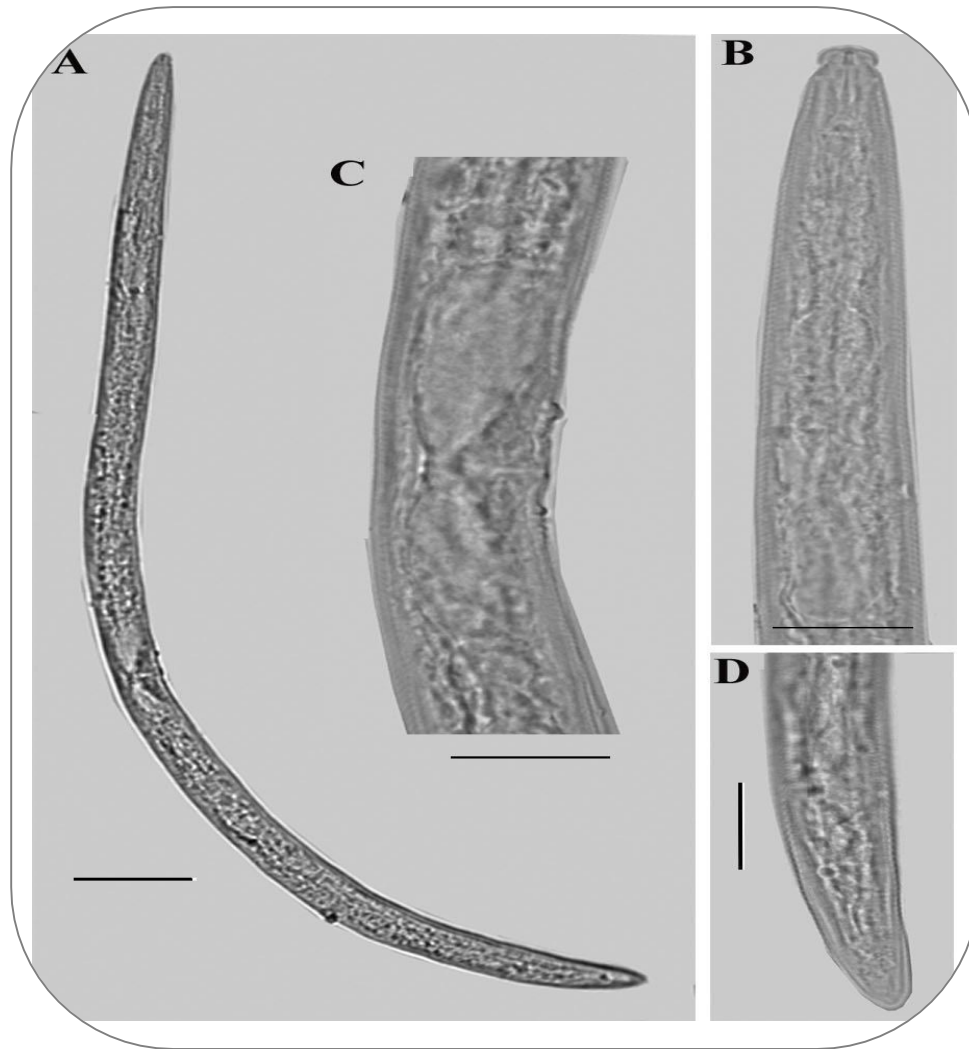


Figure 13. Light microphotograph of *Tylenchorhynchus hordi* Khan1972 (A-D). Female A. whole body; B. pharyngeal region; C. vulval region; (male) B. whole body; D. tail region.

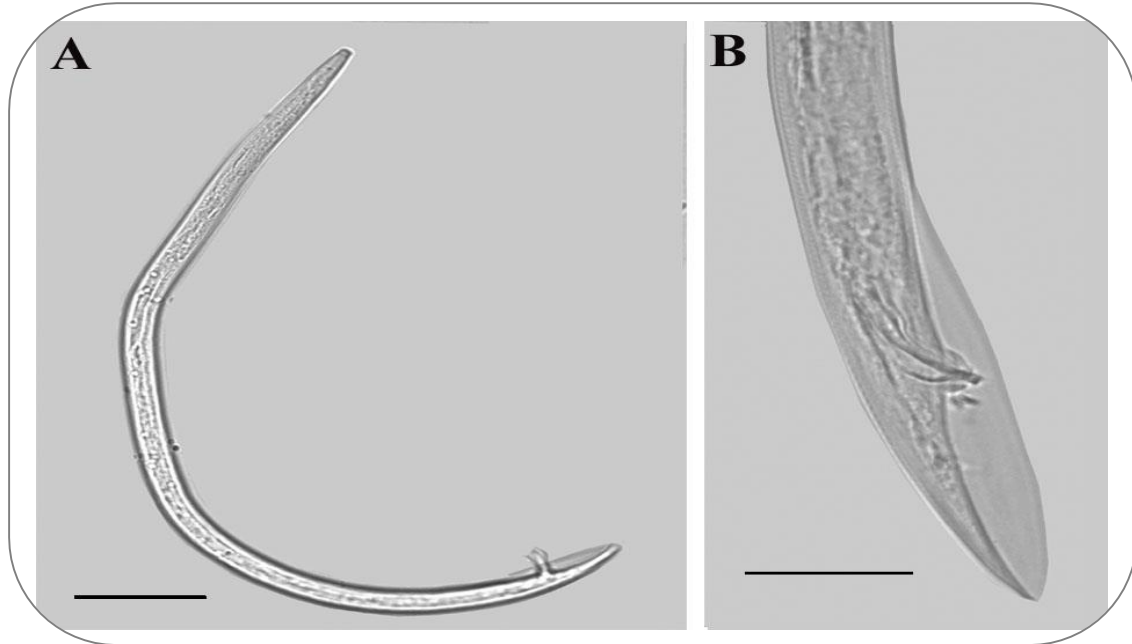


Figure 14. Light microphotograph of *Tylenchorhynchus hordi* Khan 1972 (A-B). Male: A. whole body; B. tail region showing spicule.

Seen laterally. Bursa well developed, completely enveloping tail. Phasmids anterior to middle of tail, extending into bursa.

**Remarks:** The above mentioned species was collected

from the soil around Loquat (*Eriobotroirya japonica* L.) major localities of bajaur like Arang, Hajilawang, and Gambat, closely resembles to *Tylenchorhynchus hordi* Khan 1972.

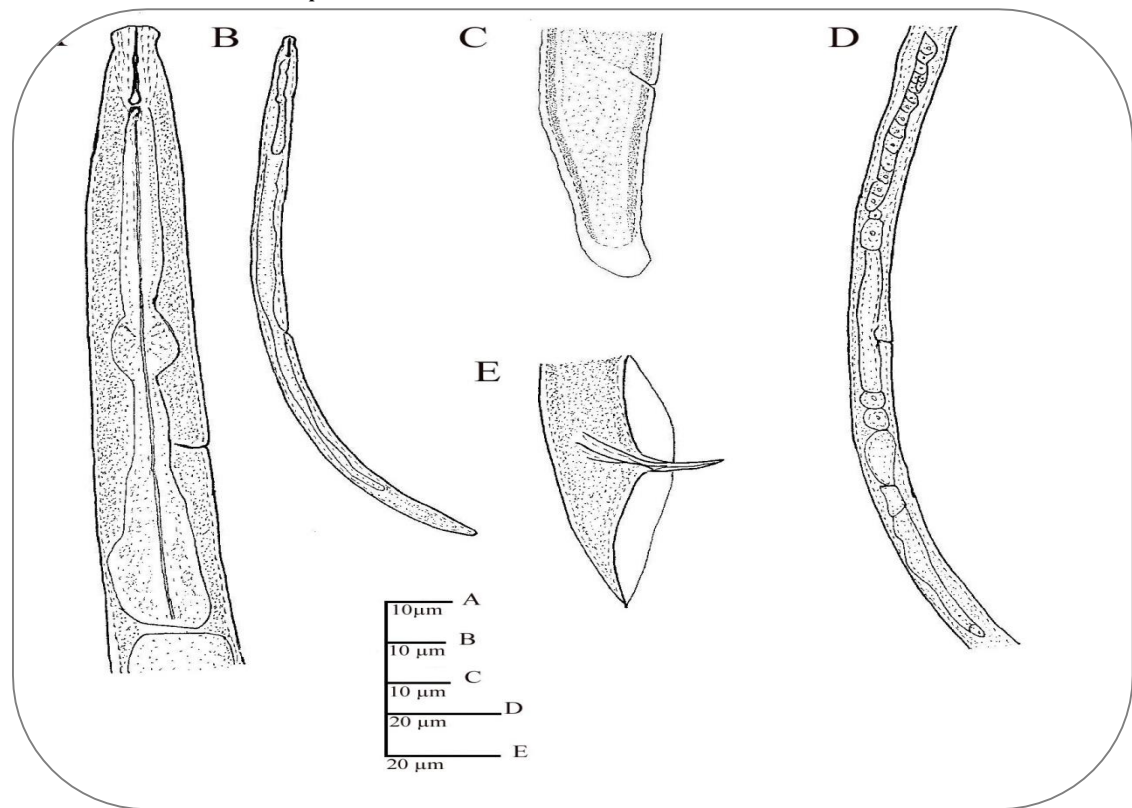


Figure 15. Light microphotograph of *Tylenchorhynchus hordi* Khan 1972 (A-F). Female A. whole body; B. pharyngeal region; C. anterior region; D. vulval region with tail; E. vulval region.

**CONCLUSION AND DISCUSSION**

In the present study seven new record species were captured *Aphelenchoides shamimi* Khera 1970; *Filenchus uliginosa* (Brzeski, 1977) Siddiqi, 1986; *Helicotylenchus africanus* (Micoletzky, 1916; Andrassy, 1958); *H. digitiformis* Ivanova, 1967; *Psilenchus curcumerus* Rahaman *et al.* (1994); *Pratylenchus wescolagricus* Corbett, 1984; *Tylenchorhynchus hordei* Khan, 1972.

Many plant parasitic nematodes were described or re-described from all over Pakistan. The parasitic genera viz., *Aphelenchoides* Fischer, 1894, *Filenchus* (Andrassy, 1954), *Helicotylenchus* Steiner, 1945, *Psilenchus* De Man, 1921, and *Tylenchorhynchus* Cobb, 1913 were reported from all over Pakistan but from bajore KPK above mentioned nematode genere were reported first time. One new and eighteen new records of the genus *Aphelenchoides* were reported whereas 2 new and 8 known species of the *Filenchus* were described from different regions of Pakistan. From *Helicotylenchus* 8 new and 37 known species, while 6 new records of *Psilenchus* were reported while 8 new and 21 known species of the genus *Tylenchorhynchus* were described or reported from different agroclimatic zones of Pakistan.

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