Pakistan J. Zool., vol. 51(4), pp 1309-1314, 2019. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2019.51.4.1309.1314

New and Known Nematodes Associated with Cotton Plantation in Sindh, Pakistan

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ABSTRACT

Cotton is a major crop of Pakistan after wheat and it occupies the largest area in Pakistan compared to other crops. Cotton is cultivated in Sindh on more than 1 million acres. Two popular varieties of cotton are grown in Sindh. The aim of the present study was to determine the diversity of plant parasitic and soil nematodes associated with cotton plants in Sindh. During 2017–2018 extensive surveys were conducted at the time of cropping and at harvesting and soil root samples were collected from different fields in the cotton producing regions of five districts of Sindh *viz*., Sanghar, Mirpurkhas, Umerkot, Mityari and Tando Allahyar. The analysis of samples resulted in the identification of one new species of soil nematode *viz*., *Acrobeloides gossypii* n. sp., along with three new record species viz., *Tylenchorhynchus ewingi* Hopper, 1959; *T. crassicaudatus* Williams, 1960 and *Pratylenchus pseudofallax* Café-Filho & Huang, 1989. Description, measurements and illustrations of these species are incorporated herein.

Article Information
Received 31 October 2018
Revised 12 January 2019
Accepted 31 January 2019
Available online 01 May 2019

Authors' Contribution
AAN conducted the field survey, executed the laboratory work and identified the species. SF helped in identification of the species. NK helped in taxonomy of the species and provided technical assistance in writing results and discussion.

Key words
Plant parasitic nematodes, Soil nematodes, Cotton.

INTRODUCTION

Several fiber crops are important agriculture commodities in the subtropics and tropics with cotton being the most important one in term of total production (Starr *et al*., 2005). Because cotton is grown as cash crop, it is often grown in a monoculture system that favors the development of a nematode community dominated by one or a few parasitic species (Starr *et al*., 1993). Upland cotton *Gossypium hirsutum* L. accounts for approximately 90% of the world production. Cotton is one of the four major crops of Pakistan and accounts for 8% of the value-added in agriculture and contributes about 2% to national GPD. The two popular varieties of cotton grown in Sindh are NIAB and newly introduced Bt (genetically modified) variety (www.sbi.gos.pk/sindh.profile). Several nematode species are reported from the cotton, but have not proven to be pathogenic. *Meloidogyne incognita*, *Rotylenchulus renifomis* and *Hoplolaimus columbus* are the most frequently encountered species found associated with cotton in the world and are responsible for the significant yield losses (Starr *et al*., 2005). These species are also recorded on cotton crop in Pakistan (Zarina and Shahina, 2013). In spite of its importance as a commercial crop, very few investigations have been undertaken to evaluate the role of nematodes as pests of this crop except for few reports of nematode infection. Recently a new species of soil nematode was described from Mubarak Village (Salma et al., 2018)

MATERIALS AND METHODS

Soil samples were collected from the cotton root zone at a depth of 15-30 cm. These soil and root samples were placed in polythene packs at 4-20ºC. The packs were labeled containing basic information about host, locality and date of collection. For separating nematodes from the soil, Cobb's decanting and sieving method (Cobb, 1918) and modified Baermann's funnel technique (Baermann, 1917) were used. The nematodes were killed by delicate warming the suspension on a hot plate at 60-70ºC. Killing was additionally done by pouring of high temperature water (80-90ºC) over nematodes in a little amount of water. The killed nematodes were instantly fixied in TAF for 24 h (Courtney *et al*., 1955). Fixed samples were kept in vials. After 24 h, the samples were washed thrice with distil water, then 2 ml of 1.25% glycerine was added to the cavity and placed it in incubator at 55ºC for 5-6 days. Nematodes were mounted in a little drop of pure glycerin (anhydrous glycerol) in the focal point of a glass-slide and secured with a 19mm cover slip containing three little pieces of paraffin wax. The slide was delicately warmed on a hot plate till the wax was soften and permitted the cover slip to settle down. At the point when the wax was set the cover slip was fixed by zut with a little delicate brush. The permanent slides were labeled containing the

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0030-9923/2019/0004-1309 $ 9.00/0

details of host, locality, number of specimens of males and females and mounting date. For light microscopy the settled nematodes were prepared utilizing techniques for Hooper (1986). Photomicrographs of nematodes were made with a programmed camera connected to a compound magnifying lens outfitted with an impedance differentiate framework. Measurements were taken with an ocular micrometer and also by tracing the outline of the structure on a paper with a drawing tube attached to a compound microscope. Measurements were taken from the drawing with a ruler or curvimeter for curved lines. Illustrations were made with the help of a drawing tube attached to the compound microscope. de Man's (1884) formula was used for measurement of nematodes. Identification of nematodes was made after Siddiqi (2000).

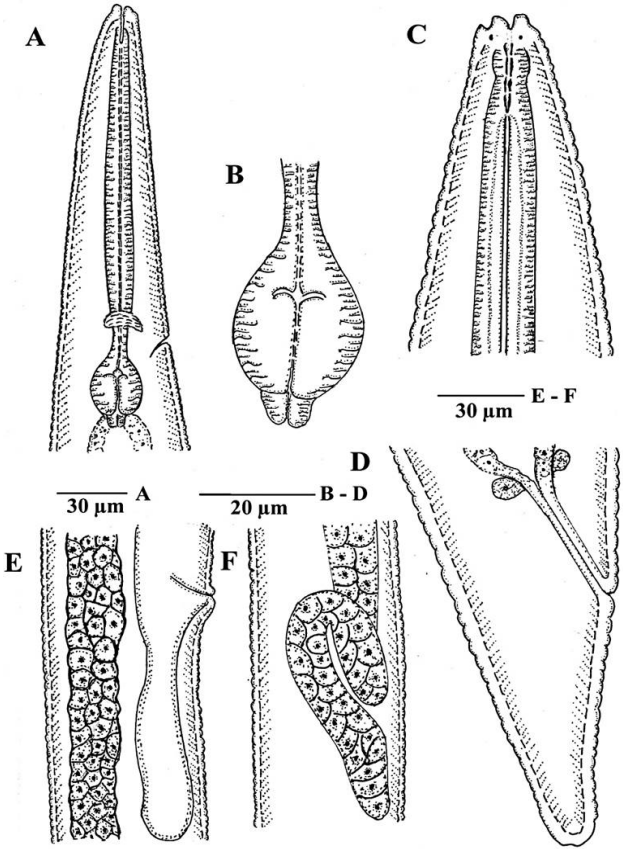

Fig. 1. Female of *Acrobeloides gossypii* n. sp.: A, pharyngeal region; B, basal bulb; C, anterior region; D, posterior region; E, vulval region showing PUS; F, reflexed ovary.

Acrobeloides gossypii n. sp. (Figs. 1 and 2)

Measurements

See Table I for measurement.

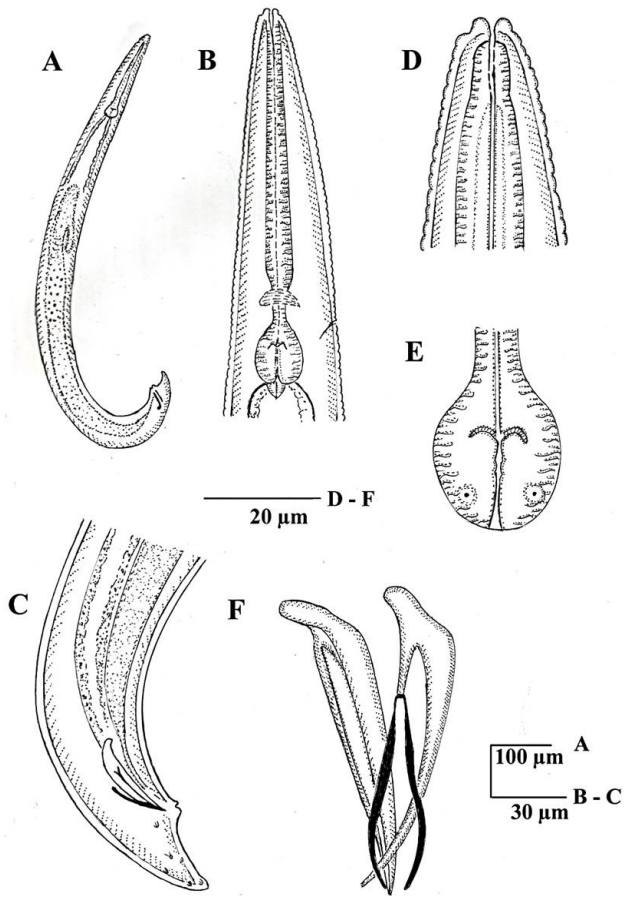

Fig. 2. Male of *Acrobeloides gossypii* n. sp.: A, whole body; B, pharyngeal region; C, tail region; D, anterior region; E, basal bulb; F, spicules and bifurcated gubernaculum.

Description

Female

Body cylindrical slightly ventrally curved upon fixation, tapering towards both ends, narrowing just behind vulva. Cuticle annulated, annuls 2-3 µm wide at mid body. Lateral field with five incisures, occupying 8-19% of the mid body diameter. Lip region with three pairs of lips. Three low and rounded labial brobolae. Amphid opening slit like stoma cephaloboid 12-16 µm long. Cheilostom with small rhabdia. Gymnostom and stegostom are narrower than cheilostom and walls appear to be sclerotized. Pharyngeal corpus cylindrical 4.3-4.6 times isthmus length. Basal bulb oval with strongly developed valves. Cardia conoid-truncated, enveloped by intestinal tissue. Excretory pore at 83-95% of total neck length at posterior part of isthmus. Deirid at the level of excretory pore, 84-87% of neck length. Nerve ring surrounding anterior half of isthmus at 82-100% of neck length. Genital system monodelphic, prodelphic ovary posteriorly directed, with double

flexure in its post vulval part. Oviduct long and tubular, spermatheca strongly developed 130-200 µm long. Post uterine sac (PUS) 0.7-1.4 times the corresponding body diameter long, distinct, vagina extending inwards to one seventh of body diameter. Rectum 0.6-0.9 times anal body width long. Rectal glands present near intestine rectum junction. Anal lips not protruding. Tail conoid to subcylindriod with 19-21 annules ventrally. Terminus smooth obliquely truncated. Phasmid just behind mid tail 25-32 µm or 53.3-59.5% of tail length.

Male

General appearance similar to female but with smaller body. Body curved upon fixation more so in posterior region. Reproductive system cephaloboid, monorchic, testis reflexed ventrally. Tail conoid ventrally curved with five pair of caudal papillae, phasmid at 60-65% tail length. Spicules paired, ventrally curved, cephaloboid, manubrium rounded, calamus conoid, small lamina wide anteriorly with dorsal hump. Gubernaculum ventrally curved, bifurcate posteriorly.

Diagnosis and relationship

Acrobeloides gossypii n. sp., is characterized by a body length of 811-1695 µm in female and 714-1392 µm in males. Lateral field with five incisures, lip region with three pairs of rounded lips and three high and conical rounded labial probolae. Pharyngeal corpus 4.0-5.2 times isthmus length. Spermatheca 56-100 long, post uterine sac 116-170 µm long or 1.1-1.7 times the corresponding body diameter long. Female tail conoid, sub-cylindrical with obliquely truncated terminus 42-55 µm; c = 16-29.2; c` = 1.1-1.9;

Table I.- Measurements (in µm) of *Acrobeloides gossypii* n. sp.

Morphological characters	Holotype (♀)	Paratypes (15 ♀♀)	Paratypes (15 ♂♂)
Body length (L)	960	117.6±351.6 (811-1695)	939.6 ± 175.9 (714-1392)
a	12.3	11.3±2.2(8.1-15.5)	14.4±1.61(11.1-16.6)
b	5.5	6.1±1.3(4.5-8.0)	5.71±0.92 (4.6-7.5)
c	19.2	22.7±4.9(16-29.2)	21.7±2.3 (19.5-27.8)
c`	1.7	1.56±0.18(1.1-1.9)	1.1 ± 0.15 (0.9-1.3)
V/T	71.2	71.9±2.1 (70.2-75)	-
Labial probolae	3	4.00±0.7(3-5)	3.5±0.5(3-4)
Width lip region	14	13.6±1.16(12-15)	12.2±1.0(11-14)
Stoma	12	16.0±3.2(12-22)	14.5±2.16 (12-15)
Pharyngeal corpus	130	135±8.1(125-150)	121±8.37 (110-134)
Isthmus	28	23.7±6.6(14-28)	17.3±1.88 (16-20)
Bulb	30	28.8±1.8(26-32.5)	26.7±2.0 (24-30)
Pharyngeal length	178	186.7±20.9(166-227.5)	165.9±13.8 (150-187.5)
Nerve ring anterior end	134	127.8±4.4(125-135.5)	125.2±10.0 (112-134)
Excretory pore anterior end	148	147.5±8.5(136-150)	136.8±4.4 (132-144)
Deirid-anterior end	152	147.4±3.3(143-152)	-
Annuli width	3	2.6±0.4(2-3)	3.5 ± 0.5 (3-4)
Cuticle thickness	4	6.7 1.05 (5-8)	4.6 ± 0.65 (4-5)
Body width: neck base	54	73.6±16.1(54-100)	51.3±3.5(46-58)
Body width: midbody	78	114.6±45.5(52-170)	71±13.1(64-87.5)
Body width: anus	40	33.9±9.7(25-50)	39.5±6.21(32-40)
Vagina	20	23.7±3.2(20-27.5)	-
Spermatheca	56	121.3±47.4(56-200)	-
Post uterine sac (PUS)	154	140.5±27.7(90-170)	-
Rectum	24	27.0±3.6(24-30)	-
Tail	50	51.8±6.92(42-55)	45.3±3.77 (40-50)
Vulva-anterior end	620	866.5±253.7(616-1225)	-
Vulva-anus	5.8	324.3±64.4(230-416)	-
Spicules	48	-	49.7 ± 6.2(38-57.5)
Gubernaculum	20	-	27.1±5.2 (24-35)

male tail conoid with minutely rounded terminus 42-57 µm; c = 19-27.8; c` = 0.9-1.31. Spicules 38-57.5 µm long and gubernaculum 24-35 µm long.

The new species resembles *A. bodenheimeri* (Steiner, 1936) Thorne, 1937 but differs from it by having more robust body (body width 52-170 µm vs. 33-40 µm); lesser a value (8.1-15.5 vs. 16.6-23.5); more posterior located vulva (70.2-75 vs. 64-69%). Post uterine sac (PUS) 1.1-1.7 vs. 9-1.1 times the corresponding body diameter long.

Type habitat and locality

Samples were collected from root zone of cotton (*Gossypium hirsutum* L.) from Matiari, Sindh, Pakistan.

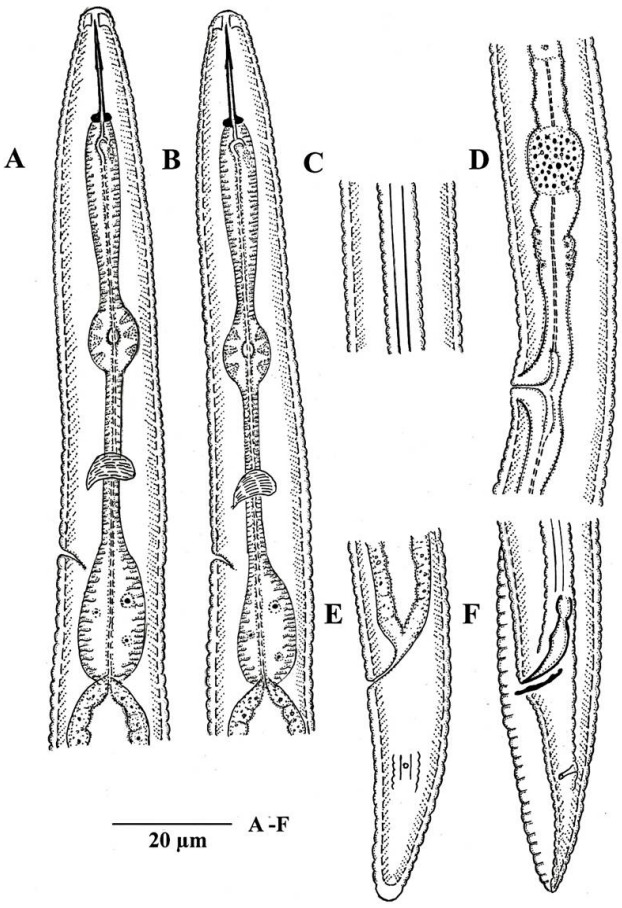

Fig. 3. *Tylenchorhynchus ewingi*, Hopper (1959): A, oesophageal region of female; B, oesophageal region of male; C, lateral field; D, vulval region; E, female tail; F, male tail.

Type material

Holotype (female): *Acrobeloides gossypii* n. sp. Slide No. 1 deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Pakistan. Paratype females (n= 25), males (n= 25) on slides No. 2-20, are deposited in the above said nematode collection.

Etymology

The species name refers to the type host cotton (*Gossypium hirsutum* L.) from where the samples were collected.

Tylenchorhynchus ewingi Hopper, 1959 (Fig. 3)

Measurements

Female (n=8)

L = 0.65 ± 0.07 (0.54–0.75) mm; a = 31.84 ± 1.94 (29.3-35); b = 4.85 ± 0.45 (4.5-5.5); c = 15 ± 1.23 (13-17); c` = 2.5 ± 0.37 (2-3); V = 55.8 ± 2.35 (53-59.3); Stylet = 19.2 ± 0.64 (18-20) µm.

Male (n=6)

L = 0.56 ± 0.02 (0.54-0.60) mm; a = 32.2 ± 1.88 (30-35); b = 4.58 ± 0.30 (4-5); c = 13.6 ± 0.36 (13-16.5); Stylet =18.5 ± 0.35 (18-20) µm; spicule = 19.1 ± 0.60 (18-20) µm; gubernaculum = 9.4 ± 0.32 (9-10) µm.

Remarks

Specimens of *Tylenchorhynchus ewingi* Hopper, 1959 collected from soil around the roots of cotton (*Gossypium hirsutum* L.) at two localities Umerkot and Sanghar, are the first record species from Sindh, Pakistan. Measurements are in close agreement with the description given by Hopper (1959). It causes stunted and retarded growth of the crop plants. Robbins *et al*. (1989) also recorded *T. ewingi* from cotton rhizosphere from India.

Tylenchorhynchus crassicaudatus Williams, 1960 (Fig. 4)

Measurement

Female (n=10)

L= 0.62 ± 0.11 (0.57-0.70) mm; a= 30.4 ± 1.77 (29-33); b= 5.3 ± 0.29 (5.0-5.7); c= 14.9 ± 0.64 (14-16); c`= 3.3 ± 0.13 (3.1-3.5); V= 55 ± 1.49 (52.5-58); Stylet = 20.2 ± 0.68 (19-21) µm.

Male (n = 8)

L= 0.65 ± 0.04 (0.58-0.70) mm; a= 29.7 ± 1.15 (27.9-31.5); b= 5. 50 ± 0.27 (4.7-5.5); c= 14.5 ± 1.21 (12.8-17); spicule= 21.8 ± 1.01 (20-23) µm; gubernaculum= 11.15 ± 0.68 (10 -12) µm; stylet= 19.8 ± 0.57 (19-21) µm.

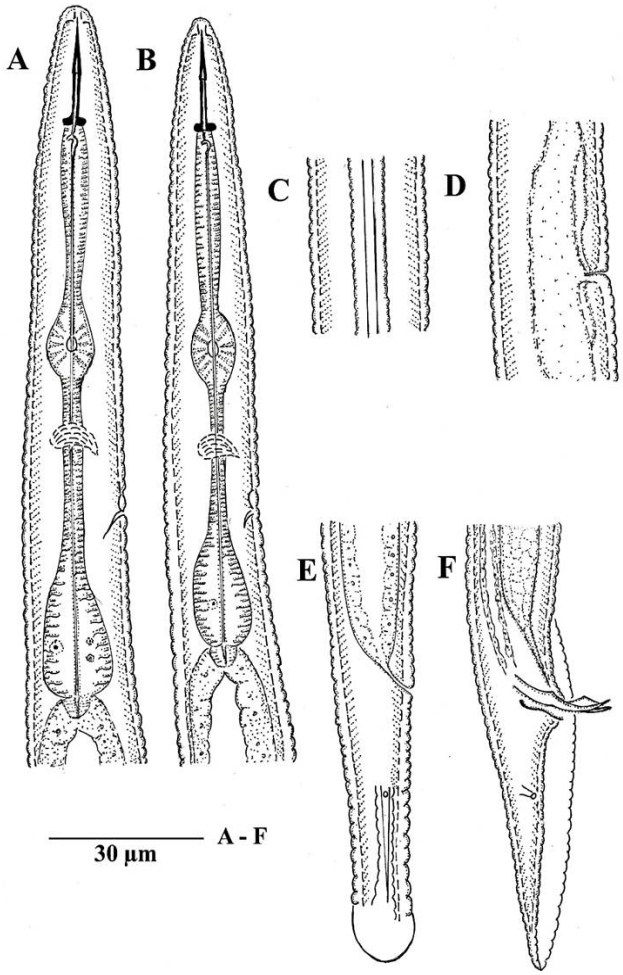

Fig. 4. *Tylenchorhynchus crassicaudatus,* Williams (1960): A, oesophageal region of female; B, oesophageal region of male; C, lateral field; D, vulval region; E, female tail; F, male tail.

Remarks

Tylenchorhynchus crassicaudatus Williams, 1960 was reported from soil around the roots of cotton (*Gossypium hirsutum* L.) from Tando Allahyar as a new record from Sindh, Pakistan. Measurements and morphological characters are quite similar to those given by Williams (1960).

Pratylenchus pseudofallax
Café-Filho & Huang, 1989
(Fig. 5)

Measurements

Female (n = 10)

L= 0.44 ± 0.05 (0.42-0.55) mm; a = 1.98 ± 9.3 (22-30); b = 3.77 ± 0.44 (3.0- 4.4); b` = 6.11 ± 0.74 (5.0-7.3); c = 18.5 ± 1.91 (15-21) c` = 2.2 ± 0.46 (1.75-3.0); V= 78.6 ± 1.20 (77.1-80.2); stylet = 15.34 ± 0.74 (14-16.5) µm.

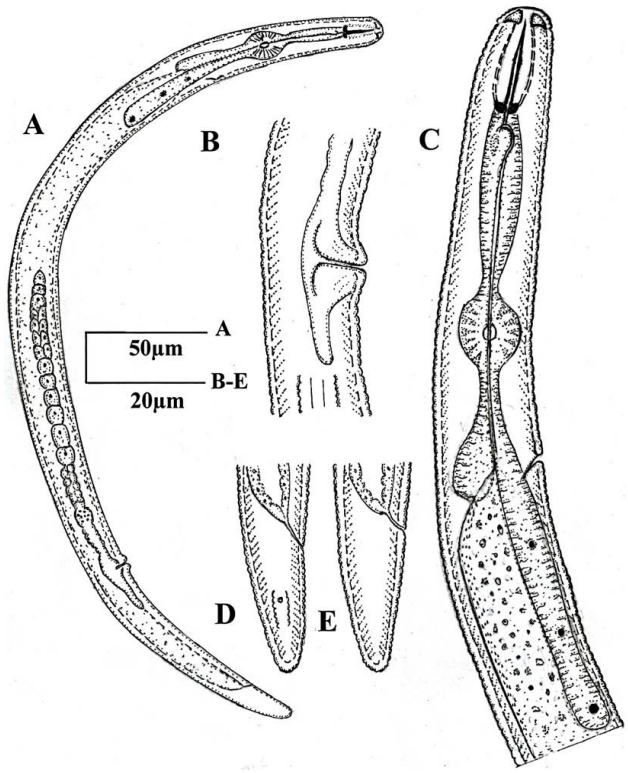

Fig. 5. *Pratylenchus pseudofallax*, Café-Filho and Huang (1989): A, whole body; B, vulval region; C, oesophageal region; D-E, tail region.

Remarks

Pratylenchus pseudofallax Café-Filho and Huang (1989) was reported as a new record from cotton growing areas of Sindh for the first time from Pakistan. This species was collected from Mirpurkas during the present studies. The measurements of these nematode specimens correspond well with the original description of Café-Filho and Huang (1989).

CONCLUSION

One new species of soil nematode *viz*., *Acrobeloides gossypii* n. sp., along with three new record species viz., *Tylenchorhynchus ewingi,* Hopper (1959); *T. crassicaudatus,* Williams (1960) and *Pratylenchus pseudofallax,* Café-Filho and Huang (1989) identified from this survey. This study will help further for the management of the cotton soil nematodes.

ACKNOWLEDGEMENT

This is a part of dissertation of first author in partial fulfilment of the requirements for the degree of Ph. D.

Statement of conflict of interest

The authors declare no conflict of interest.

REFERENCES

Baermann, G., 1917. Enine Methode Zur Auffunding Von Anklyostomom (Nematoden) Larven in Euproben Geneesk. *Natuurkundig Tijdschr. Nederl. Ind*., **57**: 131-137.

Cafe`-Filho, A.C. and Huang, C.S., 1989. Description of *Pratylenchus pseudofallax* n. sp. with a key to species of the genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae). *Rev. Nêmatol.*, **12**: 7-15.

Cobb, N.A., 1918. *Estimating the nema population of soil*. Agric. Tech. Circ. U.S. Dept. Agric., pp. 48.

Courtney, W.D., Polley, D. and Miller, V.L., 1955. TAF, an improved fixative in nematode technique. *Pl. Dis. Rep.*, **39**: 570-571.

de Man, J.G., 1884. The nematodes of the Dutch fauna living free in the pure earth and in the sweet water: A systematic-faunistic monograph. Vol. VI. Forgotten Books, London. pp. 206.

Hooper, D.J., 1986. Handling, fixing, staining and mounting nematodes. In*: Laboratory method for work with plant and soil nematodes* (ed. J.F. Southy). Ministry of Agricultural Fisheries and Food, London, pp. 59-80.

Hopper, B.E., 1959. Three new species of the genus *Tylenchorhynchus* (Nematoda: Tylenchida). *Nematologica*, **4**: 23-30. https://doi.org/10.1163/187529259X00336

Robbins, R.T., Riggs, R.D. and von Steen, D., 1989. Phytoparasitic nematode surveys of Arkansan cotton fields. *J. Nematode Suppl.*, **21**: 619-623.

Salma, J., Saima, M., Nasira, K. and Shahina, F., 2018. Description of *Aulolaimus mubarakvilli* the new species (*Nematoda: Aulolaimidae*) with observation on Heterodorus longidens from Pakistan. *Pakistan J. Zool.,* **50**: 325-328.

Siddiqi, M.R., 2000. *Tylenchida, parasites of plant and insects*, 2nd edition. CABI Publishing, Wallingford, UK, pp. 805. https://doi.org/10.1079/9780851992020.0000

Starr, J.L., Heald, C.M., Robinson, A.F., Smith, R.G. and Krausz, J.P., 1993. *Meloidogyne incognita* and *Rotylenchulus reniformis* and associated soil textures from some cotton production areas of Texas. *Suppl. J. Nematol.*, **25**: 895-899.

Starr, J.L, Carneiro, R.G. and Ruano, O., 2005. Nematode parasites of cotton and other tropical fiber crops. In: *Plant parasitic nematodes in subtropical and tropical agriculture* (eds. M. Luc, R.A. Sikora and J. Bridge). CABI Publishing, Wallingford, UK, pp. 733-750. https://doi.org/10.1079/9780851997278.0733

Steiner, G., 1936. *Opuscula miscellanea Nematologica, III*. Proceedings of the Helminthological Society of Washington, pp. 16-22.

Thorne, G., 1937. *A revision of the nematode family Cephalobidae Chitwood and Chitwood 1934*. Proceedings of the Helminthological Society of Washington, 4. pp. 1-16.

Williams, J.R., 1960. Studies on the nematode soil fauna of sugarcane fields in Mauritius. 4. Tylenchoidea (partim). *Occas. Pap. Sugar Indust. Res. Inst. Mauritius,* **4**: 30.

Zarina, B. and Shahina, F., 2013. *Annotated bibliography on plant nematology in Pakistan*, 2nd edition. National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan.