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SIX GENERA OF PLANT-PARASITIC NEMATODES FROM BANGLADESH

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Abstract

The presence of plant-parasitic nematodes causing crop loss every year had been overlooked in the existing fruits, vegetables and flowers cultivation system in Bangladesh. Therefore, the study was conducted to isolate and identify plant-parasitic nematodes from soil and infected plant-parts of fruits (banana, orange), vegetables (pointed gourd, tomato and brinjal) and flower (gerbera) from the districts of Jashore, Jhenaidah, Gazipur and Sylhet during 2017-18 and 2018-19 cropping seasons. A total of 68 samples of which 20 fruit samples, 45 vegetables samples and 3 flower samples were collected and observed for nematode infestation. Baermann funnel method was used to extract the active nematodes from plant parts and Cobb's method was used for the isolation of nematodes from soil samples. Different morphological structures of the nematodes viz. esophagus, median bulb, basal bulb, intestine, reproductive structures (vulba, bursa), etc. were used to identify the nematodes. To identify *Meloidogyne* spp. from root knot of tomato, brinjal and gerbera, juvenile larvae J_2 and perineal pattern of female nematodes were used. A total of six genera of nematodes were identified and all of them were under the order Tylenchida. The nematode genera were *Tylenchorhynchus* sp., *Tylenchus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., *Pratylenchus* sp. and *Meloidogyne* spp. The root-knot nematode, *Meloidogyne incognita* was recorded for the first time in flower crop gerbera in Bangladesh.

Keywords: Plant-parasitic nematode, infestation, isolation, identification, Baermann funnel, Cobb's method, morphology.

Introduction

Plant parasitic nematodes are most neglected problem of agricultural productivity in Bangladesh. They infect flowers, fruits, vegetables and forest trees. It has been reported that the economic loss for important crops (vegetables, fruits and edible field crops) are 14% for a total of over $80 billion annually (Agrios, 2004). About fifteen thousand species of nematodes have been reported around the world. Among them 2200 species are identified as plant parasitic (Goodey *et al*., 1965). Globally plant-parasitic nematodes considered as one of the major pests of vegetable crops. Damage caused by nematodes could reach up to 30% of total production of tomato, eggplant and melon (Janati *et al*., 2018).

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In Bangladesh, several studies were conducted on the presence of plant parasitic nematodes in potato, banana, fruits and vegetables (Timm and Ameen, 1960; Mian, 1986 and Mian, 1987). In those studies, several plant parasitic nematodes were recorded and they were *Aphelenchoides, Aphelenchus, Belonolaimus, Criconema, Criconemoides, Ditylenchus, Helicotylenchus, Hemicyclophora, Hirschmanniella, Hoplolaimus, Longidorus, Meloidogyne, Paratylenchus, Pratylenchus, Radopholus, Rotylenchulus, Rotylenchus, Scutellonema, Trichodorus, Tylenchorhynchus, Tylenchulus, Tylenchus* and *Xiphinema*. Among them *Aphelenchoides* sp., *Ditylenchus destructor*, *Helicotylenchus* sp., *Meloidogyne* sp., *M. incognita*, *M. javanica*, *Neotylenchus* sp., *Pratylenchus coffea*, *P. penetrans*, *Rotylenchulus* sp., *Rotylenchus* sp., *Tylenchorhynchus claytoni* and *Xiphinema* sp. were found with rhizosphere soils of potato. Other four types of nematodes such as burrowing (*Radopholus*), spiral (*Helicotylenchus* sp.), root knot (*Meloidogyne* spp.) and lesion (*Pratylenchus*) were found in the fields of banana. In another study, some important genera of nematode were identified in banana cultivation at Joydebpur (Choudhury *et al*., 1981). They recorded *Helicotylenchus* sp., *Hoplolaimus*, *Hirschmanniella*, *Tylenchorhynchus claytoni* and *Meloidogyne* spp. associated with the root damage of banana plantations. However, no nematode infestations have been recorded yet in flower cultivation system in Bangladesh.

Considering the above facts, this study was conducted from 2017-2019 for collection and identification of plant-parasitic nematodes from commercially grown fruits (banana, orange), vegetables (pointed gourd, tomato and brinjal) and flower (gerbera) samples collected from Jashore, Jhenaidah, Gazipur and Sylhet districts of Bangladesh.

Materials and Methods

I) Samples collection

A total of 68 samples (symptomatic root and soil from rhizosphere) were collected in 2017-2019 to identify the associated plant-parasitic nematodes. Among 68 samples, 20 from fruits, 45 from vegetables and 3 from gerbera were investigated. All the fruits and vegetables samples were collected from Jashore, Jhenaidah, Gazipur and Sylhet districts (Table 1 and figure 1). Samples of gerbera were collected from floriculture section of Horticultural Research Centre (HRC), BARI, Gazipur. For each type of crop, the rhizosphere soil was taken from the suspected root infection to make a composite sample following zig-zag pattern. Two kg soil was collected from each composite sample and kept in polythene bag for identification of nematodes.

Table 1. Particulars of soil and plant samples collected for identification of plant-parasitic nematodes in different crops grown in four districts of Bangladesh

Host plant/ soil sample	Production system	Location	Geographic location
Banana rhizospehere soil	Orchard	Gazipur, Sylhet	23° 59' 59.7876" N and 90° 25' 12.9828" E
Orange rhizospehere soil	Orchard	Sylhet	24° 53' 11.1696" N and 91° 52' 50.5992" E
Pointed gourd infected roots	Field	Jashore	23° 10' 14.3904" N and 89° 12' 44.7048" E
Tomato root gall and rhizosphere soil	Field	Jashore	23° 10' 14.3904" N and 89° 12' 44.7048" E
Brinjal root gall and rhizosphere soil	Field	Jhenaidah	23° 38' 1.8276" N and 89° 4' 0.6816" E
Gerbera root gall	Experimental field	Horticultural Research Centre, Gazipur	23° 59' 20.4504" N and 90° 25' 5.4012" E

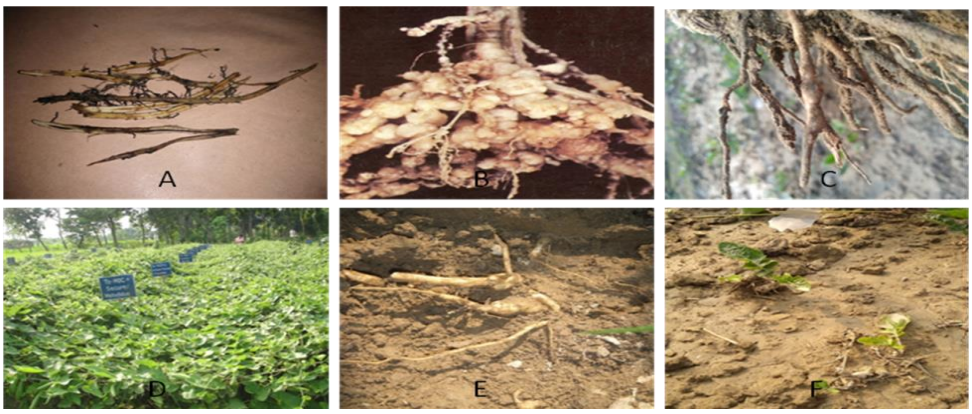

Fig. 1. Plant-parasitic nematodes infected plant samples. A) Nematode infected banana sample B) Root-knot nematode infected tomato sample C) Root-knot nematode infected gerbera sample D) Pointed gourd field E) Root-knot infected pointed gourd sample F) Root-knot nematode infected gerbera field.

II) Nematode extraction

a) Infected roots: The Baermann funnel method was used for the extraction of active nematodes from infected plant samples. The collected plant samples were cut into pieces of about 1 cm size. Root sub-samples (10 g) were wrapped with cheesecloth and formed a ball shaped structure. A clean funnel was placed in a

stand filling with water until it reaches up to 1 cm below the rim. Bubble formation inside the tubes was avoided and the clips were closed well. Samples were submerged in water of funnel for 48 hours. Active nematodes were moved to the end of the rubber tube leaving the debris behind. Syracuse dish was used to collect the nematodes from the rubber tube for identification under stereo and compound microscope.

b) Rhizosphere soil: Cobb's decanting and sieving method was used for the isolation of nematodes from soil samples (Van, 2006). The sample (maximum 100 g) was stirred in a water-filled beaker. After heavy particles had settled down, the nematode suspension was poured and sieved. Sieving was carried out with a series of sieves of decreasing mesh size i.e 500 µm, 350 µm 100 µm and 45 µm so that nematodes of different sizes could be collected separately.

III) Sample preparation for microscopic observation and identification

The following simple method was used for permanent slide preparation to identify the nematodes (Ryss, 2003).

a) Killing: A drop of 10 µl distilled water was placed at the bottom of one 0.5 ml tube. Alive nematodes were transferred in the tube with the needles. Another Eppendorf tube containing 0.5 ml 4% hot formalin was pour down in the water containing tube. The tube was closed and was shaken to prove the nematodes were not attached to the wall.

b) Fixation: After killing the nematodes, the tube was placed in water bath at 80°C for 30 min. After fixation, the nematodes were placed at room temperature**.**

c) Processing in glycerin: After reaching the tube in room temperature, the content was shaken and transferred to a glass container. The nematodes were picked out and transferred to a glass slides containing a drop of glycerin and distilled water in a proportion of 1:20. Then the slides were placed in a hotplate at 70°C for 15 min.

d) Slide preparation: The nematodes were transferred from hotplate to a drop of pure glycerin on a glass slide. A cover slip was placed over the glass slide. The border of glass slide was sealed with paraffin for preserving the slide to observe in future.

IV) Perineal pattern sample preparation for compound microscopic study

Galled roots of brinjal, tomato and gerbera flower were gently washed with tap water and were placed in 0.9% NaCl solution. Egg masses were collected using needle and placed in Eppendorf tubes under a stereomicroscope. Nematodes were dissected under compound microscope. The female nematodes were transferred

to a small drop of 45% lactic acid in plastic petri-dishes. The female body was pushed out in the solution, so that it could be held in the surface tension. A dissecting needle was used to remove the posterior part of female nematode and the cuticle was trimmed in a square pattern in the center. The body was transferred in a glycerol solution. This process was done for up to ten patterns for the same *Meloidogyne* species. Finally, the slide was covered with cover slip and sealed with nail polish (Sasser *et al*., 1983).

V) Nematode identification

After collecting the nematodes in both Baermann funnel and Cobb's extraction methods, they were observed under both stereo and compound microscopes. Most of the measurements were recorded by ocular micro meter at X40 objective. This morphological identification was done based on the book 'pictorial key to the genera of plant-parasitic nematodes' (Mai and Lyon, 1975). Identification of plant-parasitic nematodes was based on morphological characters of nematodes such as second stage juveniles J2, body length, perineal pattern, head and tail, excretory pore, dorsal esophageal gland, spicule, median bulb, basal bulb, intestine, reproductive structures (vulba, bursa).

Results and Discussion

Six species/genera of plant parasitic nematodes were identified from 68 samples collected from the infected roots and soil rhizosphere of banana, orange, pointed gourd, tomato, brinjal and gerbera samples from Gazipur, Sylhet, Jashore and Jhenaidah districts (Table 2). They were *Helicotylenchus* sp. (spiral nematode), *Hoplolaimus* sp. (lance nematode), *Pratylenchus* sp. (lesion nematode), *Tylenchus* sp., *Tylenchorhynchus* sp. (stunt nematode) and *Meloidogyne* spp. (root-knot nematode). *Pratylenchus* sp. was the key nematode in orange and banana orchard whereas for brinjal and tomato it was *Meloidogyne javanica* and *Hoplolaimus* sp. as supported by the previous findings of Bahadur (2021). *Helicotylenchus* sp., and *Tylenchorhynchus* sp. were present only on the rhizosphere soil of banana orchard. Presence of these two nematodes species on banana orchard confirms the findings of earlier researchers (Choudhury *et al*., 1981). The *Tylenchus* sp. was only present in pointed gourd field. In addition, *Meloidogyne incognita* was isolated only from the gerbera root samples. There was no previous record of plant parasitic nematode *M. incognita* in gerbera. The finding of the present study was supported by the research report of India (Meena *et al*., 2015). They reported the presence of *Meloidogyne incognita* in gerbera. This nematode caused prominent root galls in the infection site and reduced both quality and quantity of gerbera in the field.

Table 2. Particulars of plant-parasitic nematode genera identified from different crops of four districts of Bangladesh

District/ Location	Host plant	Production system	Identified genera from soil sample	Identified genera from plant root sample
Gazipur	Banana	Orchard	*Tylenchorhynchus* sp., *Pratylenchus* sp., *Helicotylenchus* sp.	-
Sylhet	Banana	Orchard	*Tylenchorhynchus* sp., *Pratylenchus* sp., *Helicotylenchus* sp.	-
Sylhet	Orange	Orchard	*Pratylenchus* sp.	-
Jashore	Pointed gourd	Crop field	-	*Tylenchus* sp.
Jashore	Tomato	Crop field	*Meloidogyne javanica, Hoplolaimus* sp.	*Meloidogyne javanica, Hoplolaimus* sp.
Jhenaidah	Brinjal	Crop field	*Meloidogyne javanica, Hoplolaimus* sp.	*Meloidogyne javanica, Hoplolaimus* sp.
Horticultural Research Centre, Gazipur	Gerbera	Research field	-	*Meloidogyne incognita*

Morphological features of nematodes:

The morphological characteristics of different nematodes isolated from different crop plants and rhizosphere soils were noted and used to identify them up to genera. The following six different nematodes genera were identified on the basis of their respective morphological characters observed under microscope.

1. *Helicotylenchus* sp.: Heat-killed nematode was C-shaped, stylet was well developed, 21-24 µm long, with 5-6 µm basal knob. Tail was slightly tapered, with anus was marked by a slight depression; terminus annulated and hemispherical in shape**.** Mail tail was similar to females, except for genital characters the spicules was present (Fig. 2).

2. *Hoplolaimus* sp. (Lance nematode): Spear knob was anchor shaped with distinct anterior projection. Tail was hemispherical and shorter than anal body diameter. The female had a short, and rounded tail (Fig. 3).

3. *Pratylenchus* sp. (Lesion nematode): Low and flattened head region was noticed with distinct head skeleton. The stylet was around 20 µm, and

moderately developed with distinct basal knobs. The esophagus had a well-developed median bulb. The female had posterior vulva and tail was tall, cylindrical to conoid. The male tail was conical with a distinct bursa that reached the tail tip (Fig. 4).

4. *Tylenchus* sp.: Female body was slender with hooked or curled tip of tail. Body was open or close C-shaped after fixation. Labial region was slightly narrower than the rest of body. Stylet was delicate, with small and rounded knobs. Tail was slightly acute to strongly curved ventrally with finely rounded to acute terminus (Fig. 5).

5. *Tylenchorhynchus* sp.: This nematode had a medium sized body. Stylet was around 20µm thin to slender, had strong knobs and cone with a long shaft. Esophageal glands were bound by a membrane into a large basal bulb, tail was round. Body was medium in size and the distance from anterior end of esophagus to median bulb less than distance from median bulb to intestine (Fig. 6).

6. Root-knot nematodes:

(a) Meloidogyne incognita-*Meloidogyne incognita* nematode was isolated from root samples of gerbera flower collected from HRC, Gazipur. The perineal region generally had an angularly oval structure with a high dorsal arch. Inverted-V shape was formed by striae in the dorsal to the tail. Striae were in distinct waves which bent towards the lateral lines. Striae were straighter with an oval appearance in ventral region (Jepson, 1987). Striae were straighter with an oval appearance in ventral region (Fig. 7. E).

From the collected soil and root samples, *Meloidogyne incognita* J2 juvenile were isolated and identified based on tail length and stylet length. The stylet of *M. incognita* was robust and distinct. The stylet length was 13.3 µm and tail length 57.6 µm.

(b) Meloidogyne javanica- *Meloidogyne javanica* root knot nematode was isolated from tomato and brinjal root samples. Distinct lateral fields formed by double incisures are typically in the perineal patterns of *Meloidogyne javanica* (Fig. 7. F). *M. javanica* had a general oval or oval to pyriform with a medium height and occasionally compressed dorsal arch in perineal regions. The tail length of second stage juvenile J2 was 55.2 µm and stylet length was 14.0 µm (Whitehead, 1968).

Fig. 2. A) C-shaped *Helicotylenchus* sp. B) Anetrior portion with stylet and basal knob. C) Spicule of male tail.

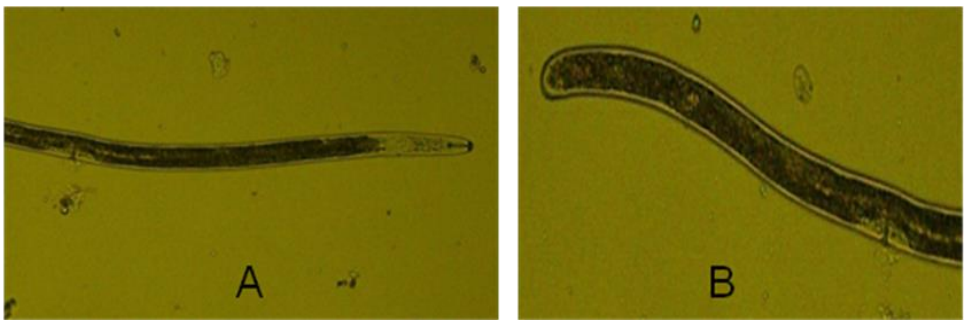

Fig. 3. A) Anterior portion of *Hoplolaimus* sp. B) Short rounded tail of female*Hoplolaimus* sp. nematode.

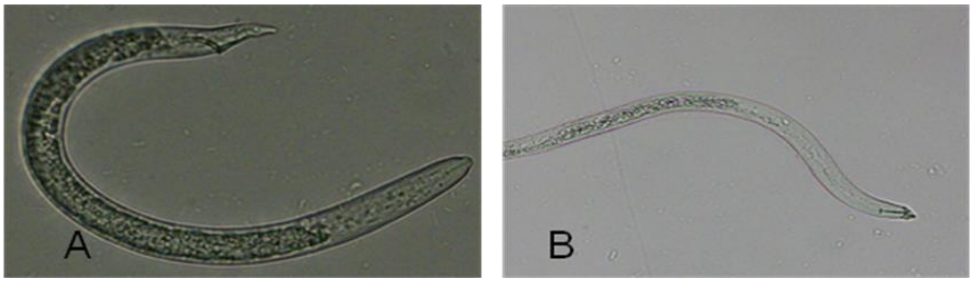

Fig. 4. A) Male *Pratylenchus* sp. with distinct bursa B) Anterior portion of female *Pratylenchus* sp.

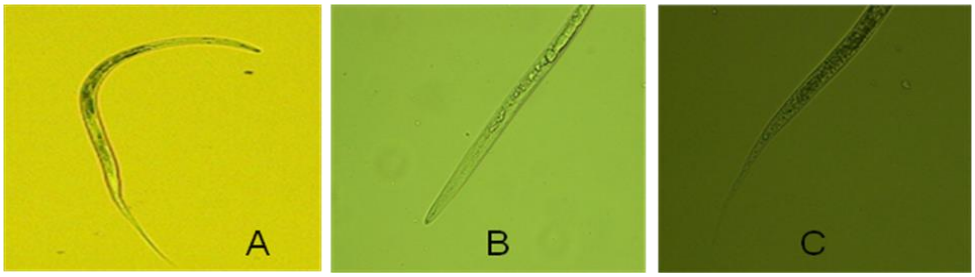

Fig. 5. A) Female *Tylenchus* sp. B) Slender female body with anterior portion C) Posterior portion.

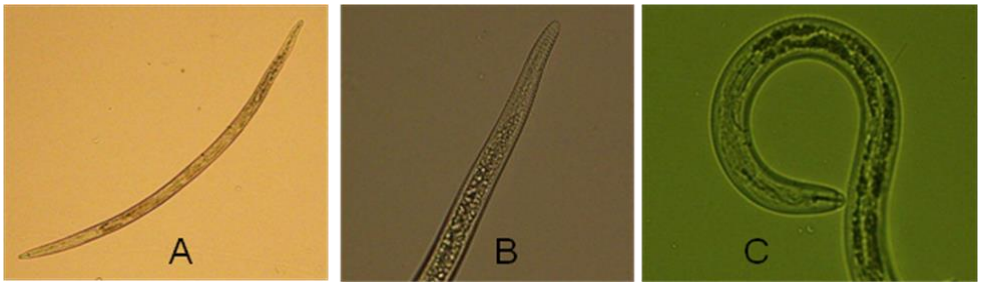

Fig. 6. A) Female *Tylenchorhynchus* sp. B) Round tail C) Anterior portion with strong basal knob.

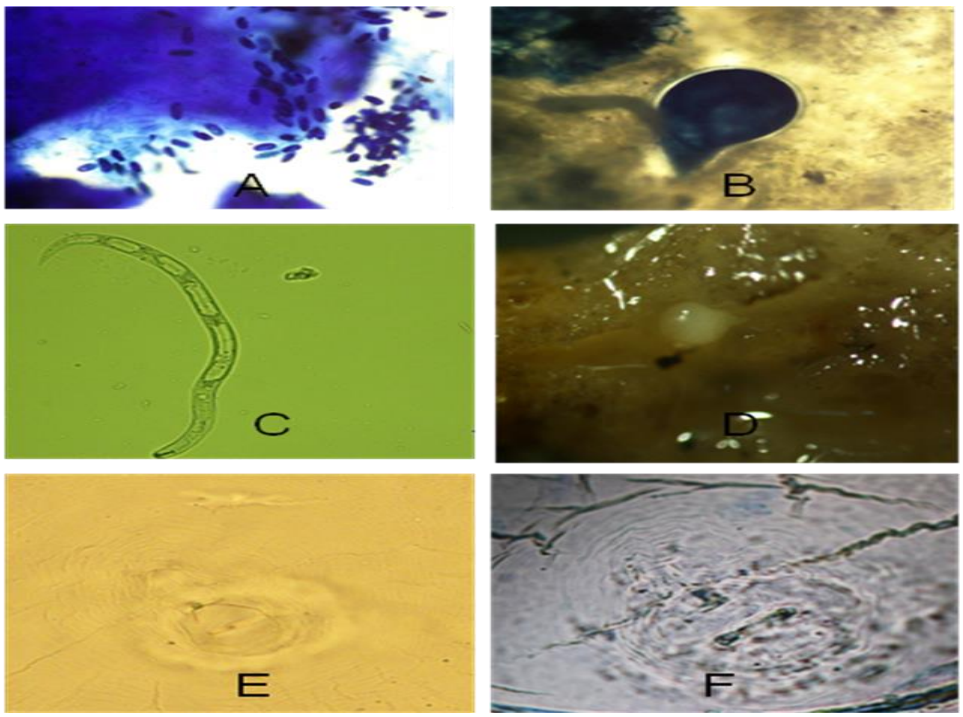

Fig. 7. A) Stained egg mass of root knot nematodes B) Immature female observed with lactophenol cotton blue C) II stage free juvenile (J2) D) Female mature *Meloidogyne javanica* nematode inside of a tomato root E) Perineal pattern of female *Meloidogyne incognita* F) Perineal pattern of female *Meloidogyne javanica.*

Conclusion

In conclusion, *Tylenchorhynchus* sp. and *Helicotylenchus* sp. of plant-parasitic nematodes were found only in banana plantations whereas, *Pratylenchus* sp. were found in both banana plantations and orange orchards. In contrast, *Tylenchus* sp. nematodes were recorded only in pointed gourd infceted root samples. *Hoplolaimus* sp. and *Meloidogyne javanica* were commonly found nematodes both in root galls and rhizosphere soil of tomato and brinjal. There were spherical galls in the roots of gerbera flower. *Meloidogyne incognita* was recorded for the first time in gerbera roots and this nematode might lower the production of gerbera flower that commericaly grown in some parts of Jashore, Bogra, Rangpur, Kushtia, Chuadanga and Jhenaidah districts of Bangladesh.

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