Pest Management in Horticultural Ecosystems
Vol. 30, No.2 pp 350-356 (2024)
DOI Number : 10.5958/0974-4541.2024.00058.0

First report of root-knot nematode, *Meloidogyne enterolobii* on watermelon in India

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ABSTRACT: Root-knot nematode, *Meloidogyne enterolobii* is a devastating species of *Meloidogyne*. This nematode was restricted only to guava. However This paper reports the occurrence of *M. enterlobii* on watermelon at Perur village, Coimbatore district, Tamil Nadu, India and it is the first record of new host of this species. Soil and root samples showed very high population (536 J_2 / 250g soil; 123±10 adult female / 5g root). Young seedlings of watermelon showed multiple compound galls on the root. Seedlings expressed stunted growth and pale yellow leaves. The species was confirmed by comparing with the original description and using molecular characterization. The morphological characters of adult females, males and second stage juveniles were measured using micrometers. Female nematodes recorded with a average body length including neck 687.8 µm; body width 425.3 µm; stylet length 13.8 µm; stylet knob height 2.8 µm; stylet knob width 4.5 µm; dorsal oesophageal gland orifice 4.90 µm and the distance from the excretory pore to head end 63.45 µm. Morphometric characters of female were similar to that of native population from guava and original descriptions.

Keywords: *Meloidogyne enterolobii*, watermelon, morphometrics, guava, first report, compound galls

INTRODUCTION

Plant parasitic nematodes are a major threat to agricultural productivity. Among major plant parasitic nematodes, root-knot nematodes act as obligate sedentary endoparasites, distributed worldwide (Jones *et al.*, 2013) and pose a major threat to about 3,000 plant species (Abad *et al*., 2021). The highest global multifariousness of the genus *Meloidogyne* occurs in Asia, where 45 species have been reported (Subbotin *et al*., 2021) while fourteen species of root-knot nematodes are recorded in India. Among identified *Meloidogyne* spp. the four major species include *M. incognita, M. javanica*, *M. arenaria* and *M. hapla* which cause economic losses in major cultivable crops (Manjunadha *et al.*, 2017). Recent report indicated that *Meloidogyne enterolobii* is becoming an emerging threat to horticultural crops due to its global distribution with a wide host range including other important economic cultivable crops (Ye *et al*., 2013; Galbieri *et al*., 2020). Moreover, this nematode has an ability to reproduce on tomato genotypes carrying Mi resistance genes (Moens *et al*., 2009; Castagnone-Sereno, 2012). In India, the first report of *M. enterolobii* was by Poornima *et al*. (2016) in guava from Ayakudi village of Dindigul district in Tamil Nadu. Later, the species diversity in Coimbatore district was morphologically and molecularly confirmed and described by Suresh *et al*. (2019) and Ashokkumar *et al*. (2019). The host range of this species was restricted only to guava till 2022 in India but, its infection was recorded first time in watermelon in a village at Coimbatore distract of Tamil Nadu, India. This paper describes the morphometric characters of this species and comparison with the native population collected from guava.

MATERIALS AND METHODS

Collection of soil and root samples

A composite of soil samples was collected from one month old watermelon field situated at Perur (Latitude: 10°58'30.32"N Longitude: 76°54'55.87"E), Coimbatore district, Tamil Nadu. A composite group of soil sample of about 200g of soil and galled roots were collected from the infested field. Similarly, soil samples from *M. enterolobii* infected guava field situated at the same location was also collected for comparison and confirmation of the nematode species. Collected soil samples were processed using Cobb's decanting and sieving method (Cobb, 1918). The infective juveniles and male nematodes were extracted using Modified Baermann's technique (Schindler, 1961).

Morphological studies

The collected infective juveniles and males were examined under compound microscope and the morphological identification was done using the generic characters described in Mai and Lyon (1975). The nematodes within the infected roots were examined using Acid fuchsin – Lactophenol method (Bybd Jr *et al*., 1983). Matured adult females were visualized under

binocular stereo zoom microscope. Permanent mounts of infective juveniles, male and posterior cuticular pattern (PCP) were processed by Seinhorst method (Seinhorst, 1959). Microscopic documentation was done using inverted research microscope (Nickon Ti2 Ecllipse).

Molecular characterization

DNA was extracted from female root-knot nematodes using worm lysis buffer (WLB; 50 mMKCl, 10 mM Tris ph 8.2, 2.5 Mm $MgCl_2$, 20 µg/ ml proteinase K, 0.45 % Tween 20 and 0.01% gelatine) as described by Castagnone-Sereno *et al.* (2012). Single female nematode was picked and transferred to 1.5 ml microfuge tube. The tube containing single nematode was added with 25 µl worm lysis buffer and crushed with needle or micropipette tips. The tubes were centrifuged at 12,000 rpm for 2 minutes and supernatant were stored at -80°C for 30 min (Adam *et al.*, 2007). The extracted DNA were subjected for PCR amplification of the 28s r RNA gene using NEM 28s F 5'-CGGATAGAGTCGGCGTATC-3' and NEM 28s R5'-GATGGTTCGATTAGTCTTTCGCC-3'primers as described by (Ye *et al.*, 2015). The reaction was executed with a total volume of 25 µl reaction which contains 2.0 µl of DNA, 1.0 µM primer (Forward and Reverse), 2.5 µl of 10X buffer, 2.5 µl 200-mM of each dNTP and 2 units of Taq polymerase enzyme and made upto 25 µl. The PCR cycles are followed by initial denaturation of 94°C for 2 min, and 40 cycles of 94°C for 30 sec, 50°C for 1 min, and 72°C for 30 sec. The reaction was terminated with 72°C for 7 min. gel was viewed in an UV transilluminator and photographed using Alpha imager TM1200 documentation and analysis system (Alpha Innotech Corporation, San Leandro, California).The PCR products were sequenced at the Yaazh Xenomics, Coimbatore.

RESULTS AND DISCUSSION

Species Description

Morphometric description of mature females

Shape of the matured females varied from pear to globular and differentiable in size, prominent neck with varying size without any posterior protuberance. Head region continuous with enlarged body (Fig. 1a). Females were white in colour. The excretory pore situated near the metacarpus and slightly varying in position depending on the size of the nematode. Annules are distinct and visible, visualized at the posterior region of the nematode body. Stylet was thin and strong with conus curved slightly at the dorsal side, knobs were distinct with a slight curve at anterior part. DOGO varies from 4.1 to 5.5 µm in length from the base of the stylet. Oesophageal gland comprises of one large uninucleate dorsal gland and two small nucleated sub-ventral glands with variability in shape, size and glandular position. The glandular lobes overlaps the intestine ventrally. The measurements of matured females were listed in Table 1. Adult females recorded an average body length of 687.8 µm; body width 425.3 µm; neck length 275.4 µm; stylet length 13.8 µm; stylet knob height 2.8 µm; stylet knob width 4.5 µm; dorsal oesophageal gland orifice 4.90 µm and the distance from the excretory pore to head end 63.45 µm. Perennial pattern located at the posterior region is oval in shape with coarse and fine striae, dorsal arch moderate to high, most probably rounded in adequate specimens (Fig.1b). Lateral lines indistinct, presence of striae in lateral sides of the vulva with prominent tail tip. Though, the size of organelles were comparatively smaller it falls within the range of native guava population and original description.

Table 1. Morphometrics of mature female *M.enterolobii* of water melon and guava populations from India

Characters	Watermelon (Present population)	Guava (Present population)	Original Description (Yang and Eisenback, 1983)
Body length	663.4±68.3 (580.2-854.3)	692.4±63.1 (524.5-765.3)	735.0±92.8 (541.3-926.3)
Body width	415.0±68.3 (390.5-530.8)	435.8±63.9 (375.1-581.6)	606.8±120.5 (375.7-809.7)
Neck length	254.3±56.8 (179.6-311.9)	271.3±52.4 (224.3-395.2)	218.4±74.1 114.3-466.8)
Stylet length	15.25±1.58 (11.5-19.0)	13.7±0.6 (13.0-14.4)	15.1±1.35 (13.2-18.0)
Stylet knob height	2.18±0.48 (1.5-2.8)	2.6±0.47 (2.2-3.3)	2.4±0.26 (1.9-3.1)

Stylet knob width	4.5±0.25 (4.0-5.1)	4.7±0.31 (4.2-5.3)	4.9±0.39 (4.1-5.6)
DOGO	4.9±0.22 (4.5-5.5)	4.8±0.4 (3.6-5.5)	4.9±0.78 (3.7-6.2)
Excretory pore to head end	60.3±4.8 (54.3-65.3)	64.8±4.2 (55.1-69.3)	62.9±10.5 (42.3-80.6)
Vulval length	29.0±1.50 (25.0-32.3)	25.8±1.3 (22.9-28.3)	28.7±2.0 (25.3–32.4)
Vulva anus distance	20.4±1.0 (18.2-20.7)	22.4±1.3 (19.1-25.3)	22.2±1.8 (19.7-26.6)
A	1.2±0.1 (1.1-1.7)	1.5±0.1 (1.1-1.8)	1.2±0.2 (0.9-1.9)

Figures in parenthesis are value range

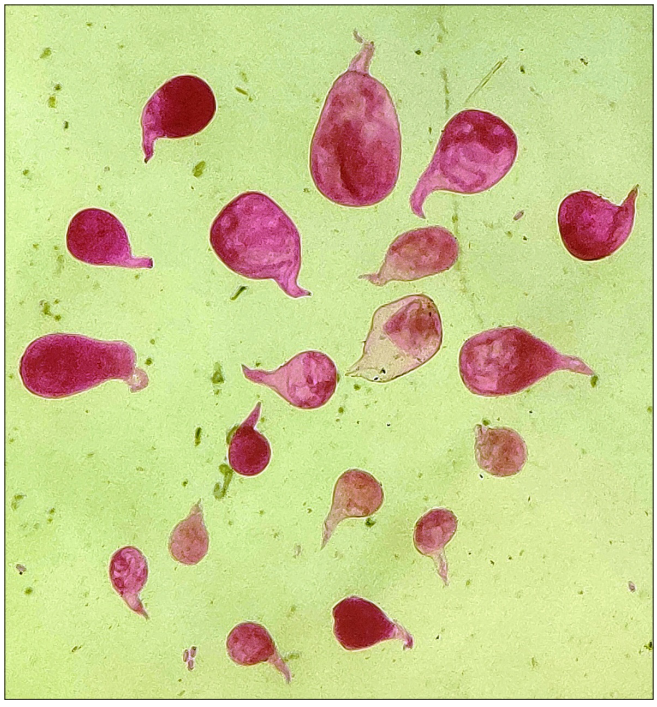

Fig.1a. Adult female nematode

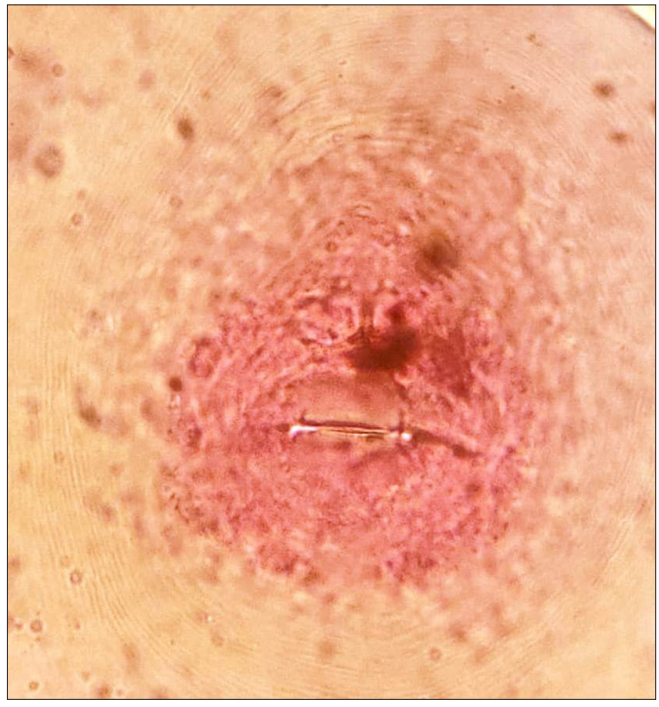

Fig.2. Posterior cuticular pattern of female

Male nematode

Males were vermiform in shape with long, slender and transparent body, tapering towards both the ends. Head region was slightly offset with moderate cephalic framework whereas the tail end was more rounded. Presence of strong and robust stylet, straight conus with cylindrical shaft with round and bigger knobs was recorded. Amphidal openings were slit like without annulations, whereas distinct annulations were present in body region. In some specimens, each knobs divided into two by a groove and the DOGO distance varied accordingly. Anterior region consists of a distinct procarpus, oval to round shaped with elongated metacarpus. Average body length was 1,234.50 µm; body width 34.70 µm; stylet length 22.23 µm; stylet knob height 2.92 µm; stylet knob width 4.98 µm; dorsal esophageal gland orifice 4.60 µm; excretory pore to head end 142.65 µm; tail length 10.45 µm and spicule length 28.88 µm (Table 2).

Table 2. Morphometrics of male *M.enterolobii* malesfrom water melon and guava populations

Characters	Watermelon (Present population)	Guava (Present population)	Original Description (Yang and Eisenback, 1983)
Body length	1584.35±85.5 (1452.6-1672.4)	1214.7±176.6 (850.1-1351.9)	1599.8±159.91 (1348.6-1913.3)
Body width	41.40±3.96 (37.4-47.5)	33.1±3.1 (27.9-39.2)	42.3±3.5 (37.0-48.3)
Tail length	10.48±2.77 (7.4-14.6)	10.57±3.15 (7.5-14.9)	12.5±2.24 (8.6-20.2)
Stylet length	23.40±1.43 (21.8-25.7)	21.5±1.4 (19.6-25.8)	23.4±0.96 (21.2-25.5)
Stylet knob height	3.05±,0.47 (2.4-3.7)	3.1±0.3 (2.4-3.6)	3.3±0.33 (2.6-3.9)
Stylet knob width	5.15±0.60 (4.5-5.8)	4.7±0.3 (3.7-7.4)	5.4±0.34 (4.5-5.8)
DOGO	3.98±0.51 (3.3-4.7)	5.4±1.2 (3.7-6.5)	4.7±0.4 (3.7-5.3)
Excretory pore to head end	170.85±2.98 (166.8-174.4)	138.4±31.7 (92.4-195.9)	178.2±11.2 (159.7-206.2)
Spicule length	30.83±2.46 (27.8-34.3)	27.8±3.2 (20.1-33.4)	30.4±1.2 (27.3-32.1)
Gubernaculum length	5.85±0.49 (5.2-6.5)	7.3±0.6 (5.7-8.4)	6.2±1.0 (4.8-8.0)
Testis length	807.03±98.17 (674.2-922.8)	302.7±86.5 (230.6-436.1)	-
A	38.43±1.97 (35.21-40.53)	36.7±4.0 (30.1-42.3)	37.9± 3.2 (34.1-45.5)
C	159.65±31.87 (114.55-196.30)	114.9±41.9 (89.5-165.8)	131.6±24.1 (72.0-173.4)

Figures in parenthesis are value range

Second stage juvenile (J_2)

Second stage juveniles was vermiform and very slender. Body length was about 425.55µm; body width 14.53µm; stylet length 11.93µm; stylet knob height 1.58µm; stylet knob width 2.64µm; dorsal esophageal gland orifice 3.25µm; excretory pore to head end 83.43µm and tail length 53.85µm (Table 3).

Diagnosis of *M. enterolobii* infestation can be challenging due to morphological similarities between it and other root-knot nematode species (Blok and Powers, 2009; Castagnone-Sereno *et al.*, 2012; Min *et al*., 2012). Later, the taxonomic status was re-entered by Karssen *et al.* (2012) and concluded it as a Synonym of *M. mayaguensis* (Rammah and Hirschmann, 1988). The morphometric measurements were similar to the original description illustrated by Yang and Eisenback (1983). They have originally described the species *M. enterolobii* parasitizing Pacara Earpod tree in China. The description derived from the second stage juvenile of collected samples were similar to the originaldescription given by (Rammah and Hirschmann, 1988).

Table 3: Morphometric dimensions of *M.enterolobii* second stage juvenile of watermelon population and in comparison with guava population from India

Characters	Morphometric dimensions of J_2 of *M. enterolobii*		
	Watermelon (Present population)	Guava (Present population)	Original Description (Yang and Eisenback, 1983)
Body length	436.50±7.13 (428.6-447.3)	413.5±26.7 (375-460)	436.6±16.6 (405.0-472.9)
Body width	15.180±45 (14.7-15.9)	13.9±1.5 (12.3-17.1)	15.3±0.9 (13.9-17.8)
Tail length	51.0±1.70 (48.6-53.4)	50.1±7.0 (42.7-75.1)	56.4±4.5 (41.5-63.4)
Excretory pore to head end	91.40±2.35 (88.4-94.6)	78.9±4.3 (73.1-92.1)	91.7±3.3 (84.0-98.6)
Stylet length	11.30±0.68 (10.6-12.4)	12.1±0.4 (11.4-12.3	11.7±0.5 (10.8-13.0)
Stylet knob height	1.88±0.19 (1.6-2.1)	-	1.6±0.1 (1.9-1.8
Stylet knob width	2.68±0.22 (2.4-3.0),8.09	-	2.9±0.2 (2.4-3.4)
DOGO	3.08±0.33 (2.7-3.6)	2.8±0.3 (2.3-3.8)	3.4±0.3 (2.8-4.3)
A	28.78±0.39 (28.13-29.16)	30.0±3.2 (24.1-35.2)	28.6±1.9 (24.0-32.5)
C	8.56±0.16 (8.38-8.82)	8.3±1.0 (6.1-10.6)	7.8±0.7 (6.8-10.1)

Figures in parenthesis are value range

Molecular characterization

Nucleotide sequence of PCR product was subjected to BLAST analysis and the results revealed 90 –100% similarities with the existing *M. enterolobii* isolates available in NCBI database. These sequence result confirmed the identity of the species as *M. enterolobii*.

***M. enterolobii* infection in watermelon**

Infected plants showed yellowing of leaves and stunted growth (Fig.2). The height of plants was about 25cm±2 cm while it was about 60cm in uninfected plants. Root showed compound galls with an average of 123±10 female nematodes per 5g root. Soil samples recorded with 536 second stage juvenile (J_2) / 250g soil. Kiewnick *et al*. (2008) reported infection of *M. enterolobii* in tomato for the first time from Mexico. First report on occurrence of this nematode in cotton was recorded by Galbieri *et al.* (2020) from Brazil. Present study revealed that the root-knot gall formation on the infected roots of watermelon were almost similar as that was reported by Poornima, *et al*. (2019) from India. These reports and present investigation confirms that the *M. enterolobii* adopts itself as a strong parasite to most of the agriculturally important crops.

Fig.2a. *M. enterolobii* infected watermelon seedling

Fig.2b. *M. enterolobii* infected root

Fig.2c. Healthy seedling

CONCLUSION

Guava root-knot nematode, *M. enterolobii* has a wide host range. In India, it was recorded only in guava and found to be introduced through infected seedling. The present study reported the damage caused by this nematode in watermelon crop for the first time in India. The crop was infected at the seedling stage itself and become completely yellow with prominent root-knot galls on the root. Molecular and morphological studies of the nematode confirmed the species. The current study is a caution to farmers and nematologists that native guava population becoming a pest of other economically important crops also. Suitable management strategy have to be formulated to combat this malady.

ACKNOWLEDGEMENT

Authors express their gratitude for laboratory facilities provided by the Department of Nematology, TNAU, Coimbatore and financial support provided by the AICRP (N) scheme, ICAR, New Delhi to conduct this study.

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MS Received: 02 August 2024
MS Acceptance: 15 October 2024