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Species diversity of root knot nematodes infesting vegetable crops in Tamil Nadu

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ABSTRACT: Random surveys were carried in vegetable growing areas of Tamil Nadu to determine the occurrence of root knot nematode species in vegetable crops. Adult females were extracted from root knot galls for species identification. The posterior culticular pattern and morphological measurement results revealed that the three root knot nematode species *viz.*, *Meloidogyne incognita, M. arenaeria* and *M. javanica* were observed on vegetable crops grown in plain areas of Tamil Nadu. Whereas, *M. hapla* was recorded only in high altitude temperate region of Tamil Nadu (the Nilgris). Nematode morphometric measurements were recorded and the species identity was confirmed by comparing the measurements with original description.

Keywords: Root knot nematodes, vegetable crops, survey, posterior cuticular pattern

INTRODUCTION

Vegetables are most important horticultural crops grown worldwide. It provides good source of vitamins, minerals, proteins and carbohydrates. Several plant parasitic nematodes were reported to be associated with vegetable crops such as root knot, cyst, lesion, reniform, lance, stem and bulb nematodes. Among the plant parasitic nematodes, root knot nematode, *Meloidogyne* spp. cause major damage to vegetable crops because to its special capability to survive on several plants and apomictic type of reproduction (Perry, 2006). It is an obligate sedentary endoparasite of more than 5500 wild and cultivated plants including 226 weed species (Rich *et al*., 2008). The aim of the current investigation is to identify and characterise the root knot nematode species associated with vegetable crops grown in Tamil Nadu.

MATERIALS AND METHODS

Survey

Through random survey, root knot nematode infested root and soil samples were collected from 28 districts of Tamil Nadu. Samples were collected from the vegetable crops showing yellowing and stunting symptoms with the help of shovel. Infested plants were uprooted and 5g of roots and 500g of rhizosphere soil were collected in polybags. The samples were labelled with the particulars on the crop, village, district, date of sampling and GPS data also were recorded. These samples were brought to the laboratory and stored in the refrigerator at 4°C for further analysis.

Maintenance of pure culture

A single egg mass collected from infested root samples was transformed to a 100 ml beaker containing distilled water and kept for hatching at room temperature (27±1°C) for 4-5 days with frequent aeration using Pasteur pipette or Aquarium pump. The hatched out second stage juveniles were inoculated in 30 days old susceptible tomato var. PKM1 for raising pure culture. This culture was used for further identification and molecular characterisation.

Preparation of Posterior Cuticular Pattern (PCP)

Identification of root knot nematodes were carried out by analysing the morphology of posterior cuticular pattern of the adult females. The females were transferred into fresh lactophenol on a thick transparent polythene film to prevent the damage of microscope stage and knife or scalpel blade. The anterior part of the female was cut with scalpel blade. Body tissues were removed by light brushing of the inner surface cuticle with a nylon bristle. The cuticle was carefully trimmed all sides so that it was only slightly larger than the perineal pattern. The trimmed perineal pattern was transferred to a drop of dehydrated glycerol on a clean glass slide. A clean coverslip was placed gently over a drop of mounting media and the edges were sealed with nail polish. The slides were prepared and photographs were taken in the Leica DM 2000 LED phase contrast microscope. The measurements of juveniles and male nematode were recorded with the help of Medline microscope (Medline scientific company limited, China) and LISS view image analyser software version 6.1.4.1(Guangzhou LISS optical Instrument Company Limited). The Camera Lucida drawings of male nematode and juveniles, whole nematode, head region, tail region were drawn using mirror type Camera Lucida (Erma, Tokyo, Japan).

Table 1. Prevalence of root knot nematodes in Tamil Nadu

District	Host Plant	*Meloidogyne* spp.	Name of Village	Latitude and Longitude	Root knot index
Coimbatore	Tomato	*M. incognita*	Mathampatti	10.5719ºN 76.5155ºE	5
	Bhendi	*M. incognita*	Narasipuram,	10.5730ºN 76.5165ºE	4
	Chilli	*M. areneria*	Thondamuthur	10.9745ºN 76.8629ºE	2
Dharmapuri	Tomato	*M. javanica*	Mathampatti	10.5719 ºN 76.5155ºE	5
	Bottle gourd	*M. incognita*	Periyathappai	12.3054ºN 78.0703ºE	5
	Chilli	*M. areneria*	Eetiyampatti	12.0470ºN 78.4833ºE	2
Dindugal	Brinjal	*M. incognita*	Palani	10.4489ºN 77.5209ºE	3
Erode	Tomato	*M. incognita*	Bhavani	11.4501ºN 77.6822ºE	4
Krishnagiri	Chilli	*M. areneria*	Hosur	12.6555ºN 78.0163ºE	2
	Tomato	*M. incognita*	Hosur	12.6565ºN 78.0166ºE	4
Tiruppur	Bitter gourd	*M. incognita*	Kethanur	10.9153ºN 77.2657ºE	5
	Chilli	*M. areneria*	Kethanur	10.9157ºN 77.2660ºE	2
Ramnathapuram	Tomato	*M. incognita*	Ckmanagalam	09.4012ºN 78.7044ºE	4
	Bhendi		Ckmanagalam	09.4017ºN 78.7050ºE	5
	Chilli	*M. areneria*	Ckmanagalam	09.4012ºN 78.7044ºE	2
Salem	Tomato	*M. incognita*	Sathanoor	12.2120ºN 78.8240ºE	4
	Bhendi		Sathanoor	12.2111ºN 78.8239ºE	5
	Brinjal		Sathanoor	12.2110ºN 78.8239ºE	3
	Chilli	*M. areneria*	Thippampatty	12.0123ºN 78.7259ºE	2
Namakkal	Tomato	*M. incognita*	Akkiyampatty	11.2797ºN 78.2346ºE	4
	Brinjal		Akkiyampatty	11.2782ºN 78.2350ºE	4
	Chilli	*M. areneria*	Sendhamangalam	11.2790ºN 78.2346ºE	2
Karur	Bhendi Tomato	*M. incognita*	Vaettamangalam	13.0945ºN 80.2930ºE	5 4
	Chilli	*M. areneria*	Vaettamangalam	13.0938ºN 80.2923ºE	2

Tiruchirappalli	Tomato	*M. incognita*	Karukkamadai	10.9600ºN 78.4470ºE	4
	Brinjal		Karukkamadai	10.9549ºN 78.4430ºE	3
	Bhendi		Karukkamadai	10.9551ºN 78.4437ºE	4
	Chilli	*M. areneria*	Karukkamadai	10.9549ºN 78.4430ºE	2
Theni	Brinjal	*M. incognita*	Periyakulam	10.1188ºN 77.5480ºE	2
	Bhendi	*M. incognita*	Periyakulam	10.1192ºN 77.5483ºE	5
Villupuram	Bitter gourd	*M. incognita*	Kallakurichi	11.7387ºN 78.9609ºE	5
	Chilli				2
Thiruvannamalai	Bhendi	*M. incognita*	Vaeppur	12.2253°N 79.0746°E	4
	Tomato				3
Vellore	Bottle gourd	*M. incognita*	Anaicut	12.8801ºN 78.9882ºE	5
Nilgris	Carrot	*M. hapla*	Kenthorai	11.4819°N 76.5683°E	4
	Tomato	*M. incognita*	Kenthorai	11.4823°N 76.5687°E	3
Thirunelveli	Tomato	*M. incognita*	Killikulam	08.3528ºN 77.6622ºE	3
	Bhendi		Killikulam	08.3530ºN 77.6625ºE	5
Perambalur	Tomato	*M. incognita*	Sathanoor	11.0917ºN 78.5829ºE	4
	Bhendi		Sathanoor	11.0920ºN 78.5832ºE	5
Pudukottai	Chilli	*M. areneria*	Anivasal	10.2044ºN 78.7401ºE	2
	Tomato	*M. incognita*	Anivasal	10.2044ºN 78.7401ºE	4
	Bhendi		Anivasal	10.2046ºN 78.7409ºE	5
Thiruvarur	Brinjal	*M. incognita*	Nallur	10.6071ºN 79.4304ºE	2
Sivaganga	Chilli	*M. arenaria*	Kallal	10.0623ºN 78.7844ºE	2
	Tomato	*M. incognita*	Kallal	10.0625ºN 78.7846ºE	3
Madurai	Cucurbits	*M. incognita*	Melur	10.0433°N 78.3419°E	5
Virudhunagar	Bhendi	*M. incognita*	Rajakottai	9.4172°N 77.8350°E	4
Cuddalore	Tomato	*M. incognita*	Nellikuppam	11.7650°N 79.6631°E	4
Thiruvallur	Bitter gourd	*M. incognita*	Tirutani	13.2265°N 80.0056°E	5

Kancheepuram	Tomato	*M. incognita*	Mamandur	12.7530°N 79.6750°E	4
Tuticorin	Tomato	*M. incognita*	Palaiyakayal	8.6701°N 78.0913°E	4
Ariyalur	Bottle gourd	*M. incognita*	Devanur	11.2654°N 79.3206°E	5

Table 2. Measurements (CV %) of female nematodes (µm) in different places

Dimension	*M. incognita*			*M. arenaria*		*M. hapla*	*M. javanica*
	Trichy	Coimbatore	Dharmapuri	Namakkal	Krishnagiri	Nilgiris	Coimbatore
Body length	17.59	6.83	10.45	12.21	23.23	26.81	17.59
Body width	15.56	8.41	14.03	29.75	21.47	19.19	14.94
Length of median bulb	15.46	16.84	11.82	16.29	10.03	9.30	12.39
Width of median bulb	17.92	10.72	15.22	3.87	5.85	8.27	8.65
Vulval slit length	11.86	14.61	8.73	11.85	8.57	17.79	8.50
Vulval to anus distance	8.77	10.69	11.6	3.91	4.07	7.95	9.50

Table 3. Measurements of juvenile nematodes (µm)

Dimension	*M. incognita*		*M. arenaria*		*M. hapla*	*M. javanica*
	Coimbatore	Trichy	Coimbatore	Trichy	Nilgiri	Coimbatore
Body length	3.95	5.2	12.64	11.6	7.9	7.1
Stylet length	9.51	6.7	6.05	5.4	9.9	7.1
'a' value	9.38	9.38	6.39	6.39	9.87	8.75
width of median bulb	9.88	15.98	13.48	10.2	16.1	33.5
Tail length	8.58	9.0	6.38	3.98	9.1	11.2

Table 4. Measurements of male nematodes (µm)

Dimension	*M. incognita* Coimbatore population (µm)
Body length	28.88
Head length	13.85
Stylet length	7.22
Length of median bulb	20.89
Width of median bulb	19.41
Spicule length	15.86

RESULTS AND DISCUSSION

Extensive surveys were carried out in 56 locations growing eight different vegetable crops covering 28 districts of Tamil Nadu (Fig.1). The results revealed that the occurrence of four major species of root knot nematodes *viz*., *M. incognita, M. arenaria, M. javanica* and *M. hapla.* Based on soil types *M. incognita* population was higher in soil with high sand content when compared to low sand content. Among the species, *M. incognita* was found predominant in all the 28 districts surveyed and in the seven vegetable crops with 100 per cent frequencies (Table 1). The severity was observed in three gourds *viz*., bottle gourd, bitter gourd and ribbedgourds with a highest root knot index of 5.0, followed by bhendi (4.64). Among the species of root knot nematodes, *M. incognita* was predominant with 100 per cent frequencies in the areas surveyed. *M. javanica* was encountered in only one locality, Thondamuthur village in Coimbatore district on tomato with a root knot index of 5.0. *M. arenaria* was encountered only on chilli in 11 localities with cent per cent frequency and the mean root index of 2.0. Skantar and Carta, (2008) reported *M. arenaria* only in chilli with a low root knot index of 2.0. In the present study, chilli is the most favoured host for *M. arenaria* and the galls produced are characteristically small as observed by Skantar *et al*. (2008). However Kalaiarasan *et al.,* (2008) were observed severe galling on root and pods of groundnut caused by *M. arenaria. M. hapla* was noticed only on carrot in The Nilgris district. Carrot is the most favoured host for *M. hapla* (Sivakumar, 1994). *M. hapla* is distributed in the higher elevation of The Nilgris district on a wide host range comprising cruciferous vegetables, legumes and weeds.

Morphometrics of *M. incognita*

The stylet length of *M. incognita* females from Tiruchirapalli, Dharmapuri and Coimbatore populations showed 5.08, 5.97 and 7.68 per cent variations. Highest variations were noticed in the dimensions of median bulb *viz.,* length in Coimbatore populations (16.84 %), width in Tiruchirapalli population (17.92 %) and Dharmapuri population (15.22 %). The perineal pattern of *M. incognita* had high dorsal arch and squarish with striations closely spaced (fig 2). Lateral field was not clear and sometimes absent. The stylet length of juvenile was with minimum variant with a CV of 3.95 % and 5.25 % in Coimbatore and Tiruchirappalli populations, and the variations were high in the length and width of median bulb, which were 5.98, 9.88, 13.02 and 15.98 in Coimbatore and Tiruchirappalli population respectively (Table 3). The stylet length of male had the minimum variations of 7.22 per cent, followed by 'a' value (9.77 %) and the highest variations were noticed in the body length (28.88 %) included in table 4. All the mean values were found in close ranges with the original description. The length of vulval slit shows moderate variability, similar patterns were noticed with J_2 and males. These findings are in line with that of Kaur and Attri (2013). Eisenback (1981) and Jepson (1987) reported that combined characters of males, females and second stage juveniles will be the reliable character for précised identification. The present study also revealed that the morphometric characters of dimensions of Coimbatore, Tiruchirapalli and Dharmapuri populations of *M. incognita*, that falls in close range to that of the original descriptions made by Whitehead (1968). So that the species was confirmed as *M. incognita.*

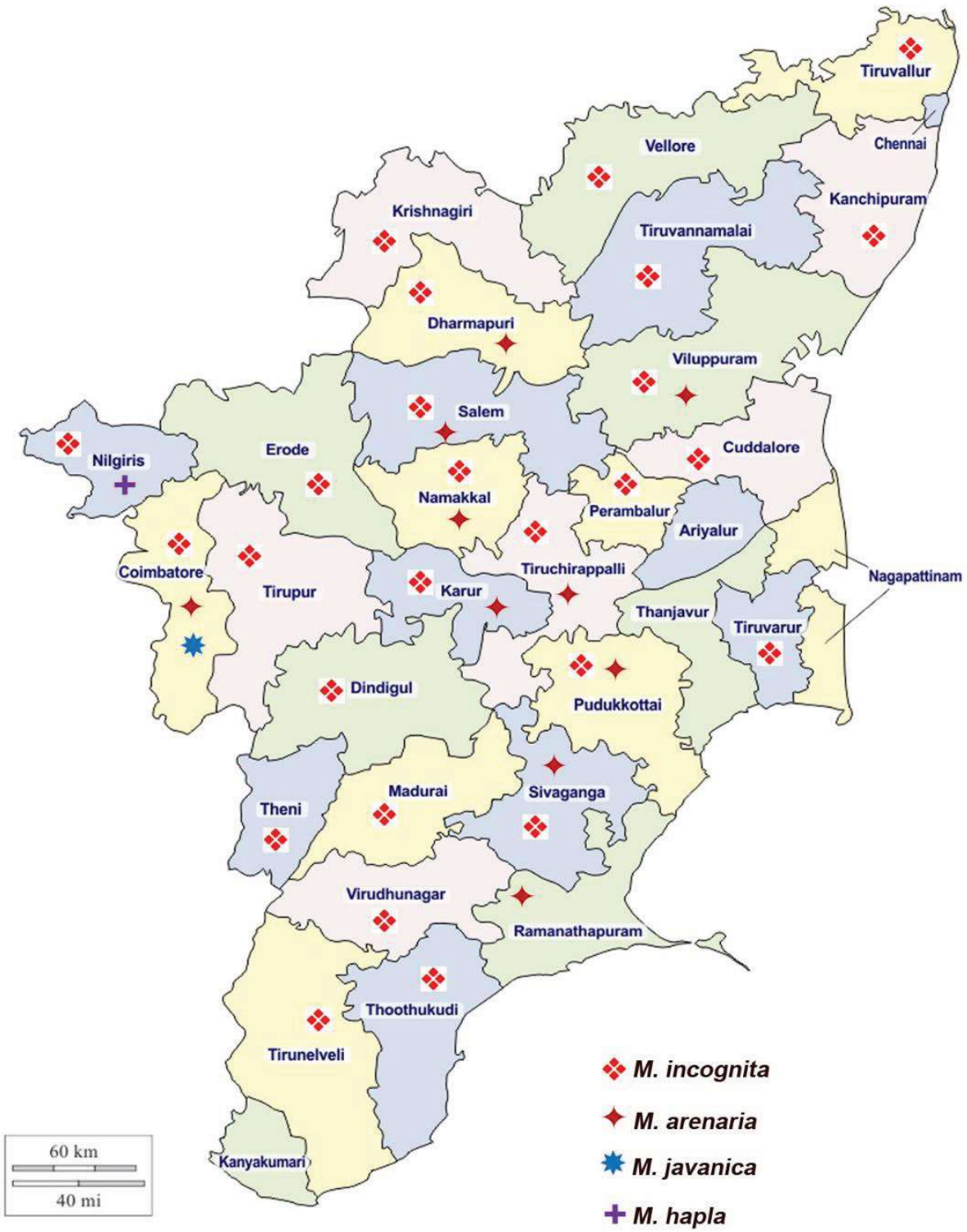
Morphometrics of *M. arenaria*

The data showed that the least variable character of female was the vulva to anus distance which was 3.91 and 4.07 per cent respectively in Namakkal and Krishnagiri populations, followed by the stylet length which exhibited a variation of 4.85 and 5.85 per cents in these two populations. *M. arenaria* has low dorsal arch, usually compressed dorso laterally (Table 2). Striations were mostly smooth. Least variability in the stylet length of J_2 was 5.4 per cent and 6.05 per cent in Tiruchirapalli and Coimbatore populations respectively. The tail length of J_2 showed a variation of 6.38 per cent in Coimbatore population and a lowest value of 3.98 per cent in Tiruchirapalli population (Table 3). High variations were noticed in terms of body length of J_2 and width of median bulb. On contrary, Cliff and Hirschmann (1985) reported that J_2 did not exhibit useful differentiating characters for the identity of the species and they did not find vast differences in the morphology of host races of *M. incognita* and *M. arenaria*. The present study revealed that a greater variations in the dimensions of median bulb and the body length of adults and in the tail length of J_2 when compared to original description by Chitwood (1949).

Morphometrics of *M. hapla*

M. hapla was circular, comprising of closely spaced smooth wavy striae with low dorsal arch. Phasmids were widely spaced (Fig. 2). Punctuations present. The data showed that the lowest variation of 6.27 per cent was noticed in the stylet length of females followed by the vulval anal distance (7.95 %) (Table 2). The vulval slit length exhibited a variation of 8.27 per cent. Highest variability was noticed with the female body length (26.81 %). Lowest variation of 7.9 per cent was noticed in the length of J_2 and highest being with the width of median bulb (16.1 %) (Table 3). The stylet length showed a 9.9 % variation within the population and it

Fig.1. Distribution of root knot nematodes, *Meloidogyne* spp. infesting vegetable crops in Tamil Nadu



showed increased values of 12.36 µm as against 9.7 µm as described in the original description by Chitwood, (1949). Likewise considerable variation was noticed in the tail length also.

The morphometric of the Nilgiris populations fall under the close range and in line with the original measurement by Whitehead (1968). The present findings of Sahoo and Ganguly (2000) compared four populations of *M.hapla* in Himachal Pradesh who have observed a high variations in terms of body length and least variability in stylets of J_2. They found a variability of less than 10 per cent and is in agreement with the present study. Brito *et al.* (2004) observed more variations in the female body length of *M. hapla* and least variabilities in 'a' value and vulval slit length

Morphometrics of *M. javanica*

M. javanica has the dorsal arch round to moderate height (Fig 2). Lateral lines clearly visible, divided the dorsal and ventral sectors. The highest variations were observed with body length (14.94 per cent). The vulval slit length was more (22.55 µm) in the Thondamuthur population compared to the original description. Lowest variations of 7.1 per cent were noticed in the stylet and the body length of J_2, followed by 'a' value (8.75 %) (Table 3). The tail length showed 11.2 % variation and the highest variations were noticed in the width of median bulb (33.5 %) within the population. There was a vast difference in the tail lengths. *M. javanica* was encountered only in one location in Coimbatore district and the morphometric characters observed on the body length, body width, stylet length, length and width of median bulb and length of vulval slit falls in close range with the original description by Whitehead (1968).

Rammah and Hirschmann (1990) studied the morphological variations between six populations of *M. javanica* and observed that the head morphology, of males and stylet morphology in the females and the posterior cuticular patterns are the useful diagnostic characters. The character which shows lowest variability can be taken as reliable for confirmation of species identity. The present study with *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica* showed a least variability in stylet length and vulval anal distance and high variabilities in terms of body length, dimensions of median bulb, and tail length. The possible reason for the high variability in body length could be that the nematodes might be in different growth stages and the variations in the median bulb may be due to the status of its pumping action at the time of fixing. On the contrary the stylet formation is completed when the J_2 is hatched out and hence possibly least variations were noticed in stylet length.

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