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Current status of root knot nematodes (*Meloidogyne* spp.) in Tamil Nadu

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Abstract
The present study was conducted to identify the current status of root knot nematodes in Tamil Nadu. Totally 20 districts were surveyed (2015-2016) for presence of root knot nematodes from various crop ecosystems and it revealed seven species *viz.*, *Meloidogyne incognita, M. javanica, M. arenaria, M. hapla, M. graminicola, M. enterolobii* (Syn: *M. mayaguensis*) and *M. naasi*. Among these species, *M. incognita* was present in all districts of Tamil Nadu that were surveyed. *M. incognita, M. javanica, M. arenaria, M. graminicola* and *M. enterolobii* were present in tropical and sub tropical regions and *M. hapla* and *M. naasi* were present in temperate regions especially in the Nilgiris. *M. incognita* populations were found mixed with *M. javanica* in some samples collected in tropical areas, while *M. hapla* populations were mixed with *M. incognita* and *M. naasi* in certain temperate regions. Incidence of *M. naasi* seems to be first report from India and it was spotted from Orange Jessamine (*Cestrum aurantiacum*) belonging to solanaceae family and grown as a hedge plant in the Nilgiris. *M. enterolobii* is an emerging problem in guava and has been reported first from Tamil Nadu in recent years and wide spread now across the Country. *Meloidogyne* spp. are usually differentiated by posterior cuticular pattern (PCP) and stylet length of second stage juveniles.

Keywords: survey, root knot nematode, *Meloidogyne* spp., posterior cuticular pattern (PCP), Tamil Nadu

1. Introduction
Root Knot Nematodes (RKNs) are the most economically important group of plant parasitic nematodes worldwide, attacking nearly every crop grown [17]. They are considered to be the most widespread and destructive plant parasitic nematodes and can cause an estimated yield loss of 25-50% over wide areas of cultivated lands [21]. The estimated yield loss on tomatoes is about 24-38% [18]. A large number of crops worldwide are affected by root knot nematodes and so far more than 100 species have been described [11]. Life cycle of *Meloidogyne* spp. can take three weeks to few months depending on environmental factors such as availability of a suitable host, temperature and moisture [21]. They release pharyngeal secretions that induce the formation of multinucleate feeding cells called giant cells. Giant cells are used as suppliers of nutrients to the growing nematode [1]. The juveniles (J2) feed, and become sedentary, then they undergo three moults (J_3, J_4 and adult) [13].
Accurate and precise identification of *Meloidogyne* species is based on a combination of several methods. Morphological characteristics and morphometric, host preferences [8], biochemical and molecular techniques are essential for confirming to identify with high precision. *Meloidogyne incognita*,
M. javanica, *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla* account for more than 95% of the occurrences of this genus and are the most widely distributed species. The impact of these species is enhanced by their wide host ranges. The most common species are estimated to be able to infect more than 5500 plant species [22].
The present study was made to record the species of root knot nematode present in Tamil Nadu and species were confirmed morphological level.

2. Materials and Methods
2.1. Soil and root samples analysis
A random survey was conducted in different districts of Tamil Nadu (Table 1) for 2015 – 2016. Roots (5g) and soil samples (200cc) were collected randomly from various crop fields from different districts of Tamil Nadu. The disease materials (galled roots) were brought to laboratory for examination.

The roots were stained in hot acid fusion lactophenol for few minutes for more detailed examination. Specific identification up to species level was done by studying the perineal pattern of the matured females. For morphological studies, infected stage juveniles, males and females were extracted from soil and roots by Cobb's decanting sieving [5] method followed by Baermann funnel techniques [3]. Photomicrograph of perineal patterns and anterior region were made by a labomed camera attached to the compound microscope. The different species root knot nematodes were identified based on the perineal pattern of the adult females as well as second stage juveniles [7].

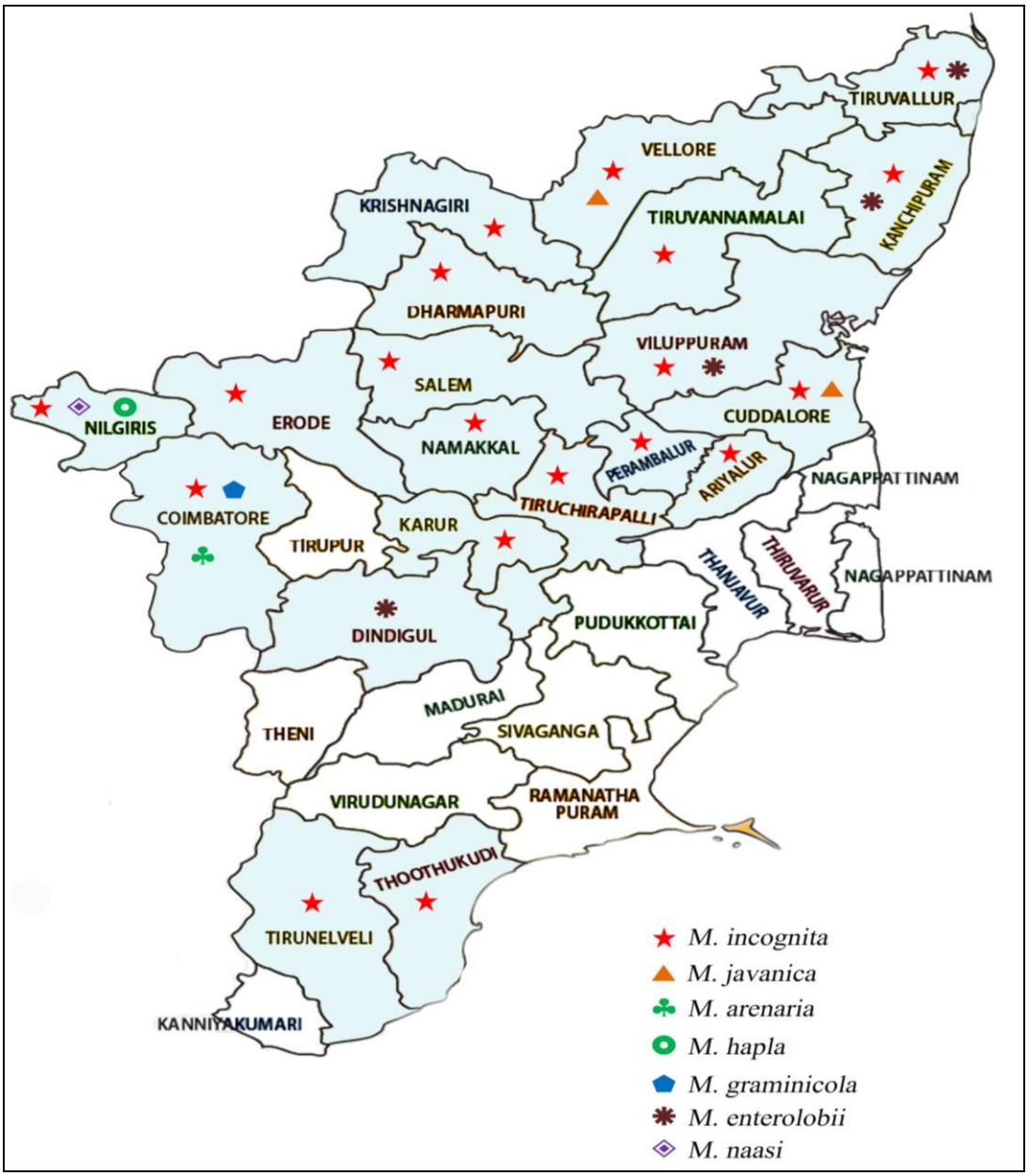

Fig 1: Map showing the distribution of root knot nematodes in Tamil Nadu

3. Results

3.1 Morphological analyses: Totally seven species of root knot nematodes were confirmed based on perineal cuticular pattern (Fig. 2) and second stage juveniles in different districts of Tamil Nadu, such as *Meloidogyne incognita, M. javanica, M. arenaria, M. hapla, M. enterolobii* (syn *M. mayaguensis*) *M. graminicola* and *M naasi.* Among these, two species were first reported in India namely, *M. enterolobii* and *M. naasi*.

3.1.1 Perineal pattern and second stage juveniles: Each and every species of root knot nematodes has different features to differentiate them. Perineal pattern is used to identify the species of root knot nematodes. The perineal pattern of root knot nematodes were detailed given as Fig. 2. Some measurement of second stage juvenile (J2) of each species root knot nematodes were described in Table. 2.

3.1.2 *M. incognita*: The perineal pattern of *M. incognita* (Fig. 2A) were characterized by the presence of high dorsal arch with squarish shape. The striae were smooth to wavy, closely spaced and varied from wavy to zigzag on dorsal and lateral side. Dorsal arch was high and rounded.

3.1.3 *M. javanica*: The perineal pattern of *M. javanica* (Fig. 2B) were rounded to oval shaped, dorsal arch round to moderate in height and sometimes flattened. Sometimes it was unique as *M. incognita* (Fig. 2A). Lateral lines or fields were clearly visible, which divided dorsal and ventral sectors. Striae were smooth to wavy, tail tip marked by an irregular whorl.

3.1.4 *M. arenaria*: The perineal pattern of *M. arenaria* (Fig. 2C) was distinguished by a low dorsal arch, round to oval which were dorso laterally compressed. Distinct lateral lines were absent, but short, irregular and forked striae marked the lateral fields.

3.1.5 *M. hapla*: The perineal pattern of *M. hapla* (Fig. 2D) were characterized by over all rounded pattern with hexagonal to flattened ovoidal shape. Punctuations were present. Some patterns formed wings on either one or both lateral sides.

3.1.6. *M. graminicola*: The perineal pattern of *M. graminicola* (Fig. 2E) was characterized by dorso ventrally oval, sometimes circular, dorsal arch low with smooth striae. Tail tip was marked with prominent, coarse, fairly well separated and disorganized striae.

3.1.7 *M. enterolobii*: The perineal pattern of *M. enterolobii* (Fig. 2F) was characterized by oval shaped striae, mostly fine. Dorsal arch were rounded to squarish, sometimes moderately high to round. Perineal patterns are similar to *M. incognita* (Fig. 2A).

3.1.8 *M. naasi*: The perineal patterns of *M. naasi* (Fig. 2G) were distinguished by exactly rounded patterns. Phasmids were prominent with fairly low dorsal arch, which were roughly circular. It was formed from broken striae that were usually well marked round the prominent phasmids.

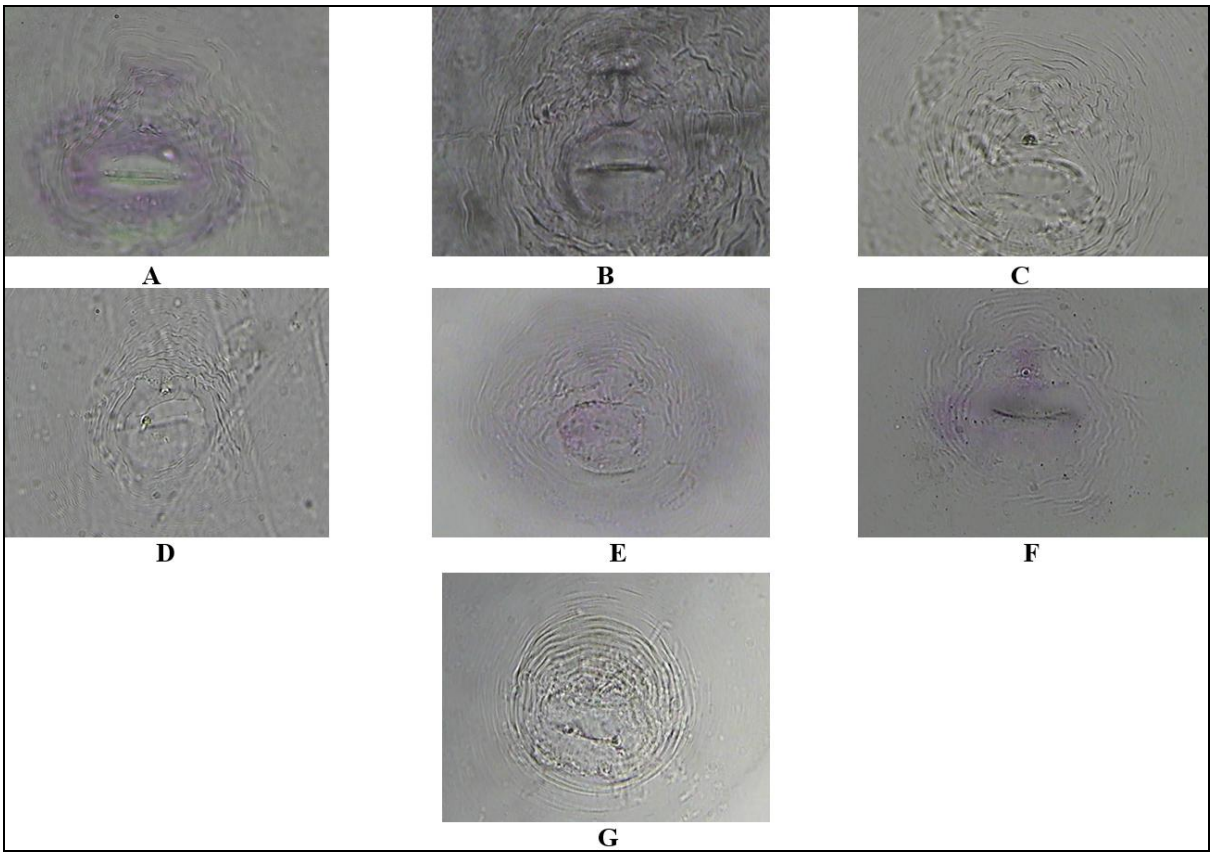

Fig 2: Species of root knot nematodes**,** A- *M. incognita*, B- *M. javanica*, C- *M. arenaria*, D- *M. hapla*, E- *M. graminicola*, F- *M. enterolobii* and G- *M. naasi*

3.2 Measurements

Apart from PCP, measurements of juveniles, head and tail portions of male and female were taken using camera lucida diagrams for further confirmation of the species. Second stage juveniles are particularly difficult to prepare for light microscopy and only specimen that are lateral and level are of value for observation and identification.

3.2.1 *M. incognita*: As per the original description the average total length of the second stage juveniles of *M. incognita* is 405 µm (346 - 463 µm) [8]. In this study, the total length of the second stage juveniles of *M. incognita* from Coimbatore 388.66 µm (350 – 411 µm) coincided with the original description. Rest of the measurement is given in Table 2.

3.2.2 *M. javanica*: As per the original description, the stylet of the female was similar to *M. incognita*, except the cone which was slightly curved dorsally [7]. The stylet length of the second stage juveniles was 11 µm (10 – 12 µm) [6]. In this study observed the stylet length of the second stage juveniles was 10.66 µm (9 – 12 µm).

3.2.3 *Meloidogyne arenaria*: Second stage juvenile stylets were similar to other species of RKNs. Tails were long and tapering. The stylet length of second stage juveniles is 11 (9.5 – 12.5) from Pollachi population were similar to original description 11.10 µm (9.5 – 12.5 µm) by [20]. Head not offset, spear knobs fairly prominent and rounded. Head smooth and marked with 1- 3 incomplete annulation.

3.2.4 *Meloidogyne hapla*: Second stage juveniles of *M. hapla* was small compare to other species of RKNs. Head cap rounded and narrow, stylet was small. Stylet length was 9.0 µm (7.0- 10.50 µm) table 2. Morphometrics for females, males and J2 were within the range of original description [4]. Measurements were similar to Chitwood, 1949.

3.2.5 *Meloidogyne graminicola*: As per the original description, the length of the second stage juveniles of *M.*

gramincola was 441 µm (415 – 484 µm), tail length was 71 µm (67 - 71 µm) and stylet length 11.4 µm (11 - 12 µm) [10]. In this study the length of the second stage juvenile of *M. gramincola* was 457.66 µm (417 – 480 µm), tail length was 65 µm (66 – 72 µm) and stylet length was 12.86 µm (11.5 – 12.5 µm) which agreed with the original description [10].

3.2.6 *Meloidogyne enterolobii* (Syn: *M. mayaguensis*): Body vermiform, tapering at both ends but more sharply posteriorly. Body cuticle with fine, distinct annulation, annules larger on posterior tail region. Head region truncate, slightly set off from body. Stylet length was 13 µm (12-13.5 µm). Other characters and measurement were similar to original description Yank and Eisenback [23].

3.2.7 *Meloidogyne naasi*: Juveniles spear noted very slender with backwardly sloping knobs. Dorsal gland opens 2-3µm behind spear base. Median bulb fusiform with vesicle like stricture similar to male. Stylet length of second stage juveniles was 12 µm (13-14.5 µm).

Table 1: Presence of root knot nematodes, *Meloidogyne* spp. in Tamil Nadu

S.no	Villages	Host	Districts	*Meloidogyne* species	Coordinates (latitude- longitude)
1	Vadakaram poondi Korakkavadi Ramanatham	Tomato Brinjal Groundnut	Cuddalore	*M.incognita* *M.javanica* *M.arenaria*	11°26'51.51"N- 78°55'47.57"E 78°54'29.93"E- 11°28'43.63"N 11°24'26.68"N- 79° 0'30.90"E
2	Velluvadi and Kaikalathur	Tomato Brinjal	Perambalur	*M.incognita*	11°28'10.37"N- 78°54'29.93"E 11°28'43.63"N- 78°51'26.55"E
3	Tindivanam Kallakurichi	Paddy Tomato Guava Groundnut Brinjal Cucumber	Villupuram	*M.graminicola* *M.incognita* *M.enterolobii* *M.arenaria*	12°14'12.78"N- 79°38'59.82"E 11°44'19.32"N- 78°57'39.27"E
4	DonBosco college, Takkolam	Brinjal Tomato	Vellore	*M.javanica* *M.incognita*	13° 1'37.09"N- 79°46'36.45"E
5	Marimangalam Perampakkam	Brinjal Tomato Guava	Kancheepuram	*M.incognita* *M.enterolobii*	13° 0'32.89"N- 79°47'9.78"E 12°54'2.62"N- 80°11'48.77"E
6	Kasuva Velliyur.	Guava Tomato Brinjal Paddy	Tiruvallur	*M.enterolobii* *M.incognita* *M.graminicola*	13°10'7.43"N-80° 1'33.63"E 13°12'51.51"N-80° 0'11.46"E
7	Mathipalayam, Thondamuthur Maruthamalai Madhampatti TNAU	Tomato Brinjal Chilli Tuberose Grapes Blacknight shade Paddy	Coimbatore	*M.incognita* *M.arenaria* *M.graminicola*	10°55'57.87"N- 76°50'29.27"E 10°34'38.39"N- 77° 2'53.19"E 11° 2'19.11"N- 76°52'3.98"E 10°58'11.39"N- 76°51'35.44"E 11° 0'44.30"N- 76°56'9.25"E
8	Sivagiri	Tomato Pomegranate	Erode	*M.incognita*	11° 7'4.33"N- 77°47'17.22"E
9	Kumaravalasu	Tomato	Karur	*M.incognita*	10°55'59.18"N- 77°46'27.03"E
10	Palani Ayakudi	Guava	Dindigul	*M.enterolobii*	10°26'56.11"N- 77°31'15.38"E 10°26'54.55"N- 77°33'6.88"E
11	Attur	Tomato Brinjal Turmeric	Salem	*M.incognita*	11°37'47.64"N- 78°10'35.02"E
12	Ooty Kuruthukuli, Nanjanadu, HRS Farm Pykkara	Potato Carrot Beetroot Broccoli Orange Jessamine (a hedge plant)	The Nilgiris	*M.incognita, M.hapla* *M.naasi*	11°24'23.09"N- 76°41'35.68"E 11°23'21.00"N- 76°38'33.79"E 11°21'59.11"N- 76°38'31.67"E 11°24'25.28"N- 76°42'32.82"E 11°28'19.58"N- 76°36'17.30"E
13	Karumanur	Tomato Chilli	Namakkal	*M.incognita*	11°27'18.14"N- 77°59'58.41"E

14	Paiyur	Tomato	Krishnagiri	*M.incognita*	12°22'10.73"N-78°13'8.88"E
15	Pennagaram and Kadathur	Brinjal Tomato	Dharmapuri	*M.incognita*	12° 8'0.28"N-77°53'48.29"E 12° 5'24.01"N-78°17'20.03"E
16	Manachanalur and Sirugamani	Paddy Cucumber	Trichy	*M.graminicola* *M.incognita*	10°54'52.14"N-78°41'57.96"E 10°53'40.50"N-78°32'1.14"E
17	Jayankondam Senthurai	Paddy Tomato	Ariyalur	*M.graminicola* *M.incognita*	11°12'51.65"N-79°21'40.55"E 11°15'18.75"N-79°10'28.58"E
18	KTC Nagar	Brinjal	Tirunelveli	*M.incognita*	8°43'1.48"N-77°46'15.45"E
19	Srivaikundam Vallanadu Killikulam	Brinjal Cucumber Tomato	Tuticorin	*M.incognita*	8°37'52.37"N-77°54'44.86"E 8°43'10.80"N-77°51'3.68"E 8°42'13.81"N-77°51'44.84"E
20	Vandavasi	Brinjal	Tiruvannamali	*M.incognita*	12°30'10.38"N-79°36'8.93"E

Table 2: Measurements of second stage juveniles of root knot nematode species.

S. No	Characters	*M.incognita*	*M.javanica*	*M.arenaria*	*M.hapla*	*M.gramnicola*	*M. enterolobii*	*M. naasi*
1	Body length	388.66 (350 -411)	499.66 (388 - 547)	490.33 (399 - 595)	3 337.66 (315 -350)	457.66 (417 -480)	448.33 (415 - 495)	432 (433-456)
2	Stylet length	11 (9 - 12)	10.66 (9 - 12)	11 (9.5 – 12.5)	9 (7 –10.5)	12.86 (11.5 -12.5)	13 (12-13.5)	12 (13-14.5)
3	Length of median bulb	9.83 (9.5 – 10.5)	12.16 (10.5 - 14)	9.83 (9.5 – 10.5)	9.23 (9.2 -9.5)	8.50 (9.0- 12.0)	9.30 (9.5 – 10.5)	8.40 (9.5-12.5)
4	Tail length	46 (43 - 50)	42 (32 - 58)	40 (45 - 68)	38.33 (34-36.5)	65 (66 - 72)	52.8 (42 - 62.5)	61.6 (56 - 65)

4. Discussion

Root-knot nematodes (*Meloidogyne* spp.) are one of the most important polyphagous nematode pests of agricultural and horticultural crops. This nematode is widespread, polyphagous and is considered economically most important for being a sedentary parasite of vascular tissues of roots. Starting with minute primary galls at the nursery stage**, c**onspicuous root galling appears after 1-2 months due to compounding of galls. The galling pattern is highly variable according to plant species and nematode species [15]. An attempt was made to record the root knot nematode species of occurring in Tamil Nadu by taking up a survey of twenty districts in various crops including vegetables, fruits, cereal, oilseed, spice and weed crops that contained galls and knots in roots.

A total of seven species of root knot nematodes were recorded from different districts surveyed randomly in Tamil Nadu which included the southern root knot nematode *M. incognita*, *M. javanica*, peanut root knot nematode *M. arenaria*, northern root knot nematode *M. hapla*, rice root knot nematode *M.* graminicola, guava root knot nematode *M. enterolobii* (Syn. *M.mayaguensis*) and barley root knot nematode *M. naasi*. Among these *M. incognita* was present in all districts of Tamil Nadu, though it possessed much variation in posterior cuticular pattern especially in its dorsal arch. Most prevalent root knot nematodes *viz*., *M. incognita, M. javanica*, *M. arenaria* and *M. graminicola* have been described by [12].

Root knot nematodes are adapted to parasitize on large number of plants and over 3000 wild and cultivated plant species are reported to be affected. Several weed species (226 species belonging to 43 families) are known to act as hosts of root knot nematode [12]. In the present study, *M. graminicola* was invariably the species observed in paddy, *M. arenaria* in groundnut, *M. incognita* in most of the tropical vegetables and spice crop, such as turmeric. The barley root knot nematode, *M. naasi* was spotted from a hedge plant in the Nilgiris grown on sides of the roads almost as a weed. The eroded soil revealed its long clusters of roots that were hanging from the raised elevations of the roads which in turn possessed obvious galls. The roots were collected, stained and observed for females and the pattern proved to be a species not so far recorded, and was identified as the barley or cereal root knot nematode, *M. naasi*. It seems to be a first report from India. Guava samples from various districts revealed presence of *M. enterolobii* (Syn. *M.mayaguensis*), yet another root knot species which is an emerging problem in most of the guava growing areas in the Country [15]. Other root knot nematode species like *M. arenaria* and *M. javanica* were found localised in particular pockets growing either groundnut or vegetables.

In the present study, carrot samples collected from the Nilgiris had infestations of northern root knot nematode, *M. hapla*. However, Nisha *et al* [13] had reported the incidence of *M incognita* on carrot from Kerala. The root-knot nematodes, *Meloidogyne* spp. are reported to be parasitic on many vegetable crops in warm humid areas of Kerala. Carrot is reported to be attacked by *M. incognita* and *M. hapla* in Jammu and Nilgiris respectively [19]; Anita and Selvaraj [2]. Narayana *et al* [14] recorded presence of *M. incognita* from cabbage roots in Thiruvananthapuram district of Kerala.

5. Conclusion

From the present study results. It is concluded that in the tropical regions, *M. incognita, M. javanica, M. arenaria, M. graminicola* and *M. enterolobii* were recorded while in the temperate regions, it was *M. hapla* and *M. naasi*. Some mixed population were found in tropical regions, such as *M. incognita* along with *M. javanica* and in temperate regions it was a combination of *M. hapla, M. naasi* and *M. incognita*. Sasser [16] reported that various *Meloidogyne* species are distributed worldwide, some occurring in the tropics, subtropics (*M. incognita, M. javanica* and *M. arenaria*) and others in temperate region (*M. hapla*). A cursory perusal of literature revealed the occurrence of *M. incognita* throughout Tamil Nadu and was the predominant species in almost all the cultivating crops and weeds. Some more species may be found in Tamil Nadu in unexploited areas and crops like tea, coffee and other plantation crops.

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