

Identification of root knot nematode in thippali

■ SEENA R. SUBHAGAN* AND SUSANNAMMA KURIAN¹

Department of Vocational Higher Secondary, Government of Kerala, KERALA (INDIA)

¹Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara, THRISSUR (KERALA) INDIA

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ABSTRACT

Thippali, *Piper longum*, is an important medicinal plant belonging to the family Piperaceae. An increase in root knot nematode attack was observed in thippali growing plots in Kerala. But the nematode species has not been identified. The study was conducted to identify the root knot nematode attacking thippali at Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. The study was undertaken by collecting soil and root samples from thippali growing plots already infested with root knot nematode. The species of root knot nematodes were identified by the perineal pattern of white females collected from root galls. The white females collected from the infested roots were sent to Department of Nematology, Indian Agricultural Research Institute, New Delhi for species identification. The species of root knot nematode attacking thippali was identified as *Meloidogyne arenaria*. This is the first report on attack of *M. arenaria* on thippali from India.

*Corresponding author:

Email : seenarubhagan@gmail.com;
dhalindharan@gmail.com

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INTRODUCTION

India, a treasure house of medicinal plants, has one of the oldest, richest and diverse cultural traditions associated with the use of medicinal plants. In recent years there has been an increased trend in cultivation of medicinal and aromatic plants. Thippali, *Piper longum* is an important medicinal plant belonging to the family Piperaceae. It is most important species of genus *Piper* after black pepper (*Piper nigrum* L.) and betel vine (*Piper betle* L.) and perhaps the first pepper to reach

Mediterranean and was once graded superior to black pepper by Greeks and Romans. It is a slender aromatic climber with perennial woody roots and is distributed along the watercourses and over shoal lands in Assam, Kerala and Karnataka. The unripe female spikes and to a smaller extend, root and thick basal stems contribute the commercial produce. The medicinal use of dry spikes and root of *Piper logumin* Ayurvedic system of medicine have been described in many studies (Kirtikar and Basu, 1935; Suseelappan, 1991; Hussain *et al.*, 1992 and Sivarajan and Indira, 1995). In Ayurveda and Unani

medicines, it is used against bronchial asthma, insomnia, jaundice, a stimulant, alternative and an expectorant and is administered for chronic bronchitis and asthma. In Ayurveda, black pepper, long pepper and ginger are collectively called as 'Trikatu'. The capacity of *Trikatu* to increase the bio availability of other drugs was reported by Atal *et al.* (1981); Manavalan (1990) and Johri and Zutshi (1992). *Meloidogyne* spp. commonly known as root-knot nematodes, are the most important enemies causing wreaking havoc to agricultural crops. They are sedentary endoparasites and produce root knots or galls of various sizes and shapes. In India, four major species of root knot nematode *viz.*, *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are to be widely distributed, attacking a wide range of agricultural crops (Dasgupta and Gaur, 1986; Jain and Hasan 1995 and Khan *et al.*, 1994). The presence of phytoparasitic nematodes in forty six medicinal plants in Nilgiris, Tamil Nadu were reported (Sivakumar and Vadivelu, 1997). Root knot nematodes cause aerial symptoms like yellowing, chlorosis, wilting and reduction in growth and underground symptoms like gall formation. The broad leaved plants may virtually show day time wilting. Galls are seen through the entire length of roots and may contain many nematodes and as a result the growth of roots is arrested. The International *Meloidogyne* Project (IMP) had identified four races of *M. incognita* and two races of *M. arenaria*. Races 1, 2 and 3 (out of four races) of *M. incognita* and race 2 (out of two races) of *M. arenaria* are reported to be widely distributed in continents and sub continents of the world (Sasser, 1982). Among the *Meloidogyne* spp., *M. arenaria* is widely distributed in various parts of India. A study of plant parasitic nematodes associated with medicinal plants in Kerala revealed the occurrences of root knot, burrowing and spiral nematodes in rhizosphere of the majority of medicinal plants (Sheela *et al.*, 1998). Thirteen new species of *Meloidogyne* have been described in the last decade, totalling more than 90 recognized species in the genus (Hunt and Handoo, 2009 and Moens *et al.*, 2009). Because distinct species of root knot nematodes attack different plant cultivars and resistance is species or cultivar specific and only effective against one or few nematode species or races, successful control of root knot nematodes requires accurate identification of nematodes present in the field (Eisenback, 1982). Species of *Meloidogyne* can be identified based on female adult morphology, including

head structures, perineal pattern, and stylet (Eisenback *et al.*, 1980). The perineal pattern is a valuable morphological feature used in the species identification of the genus *Meloidogyne* (Chitwood, 1949; Eisenback *et al.*, 1980 and Hirschmann, 1985). Nematodes of *M. incognita* were isolated from the roots of hot pepper plants, *Capsicum annum* L., from the greenhouse situated in Portorož at the Adriatic coast, Slovenia by Širca *et al.* (2004). The nematode was morphologically identified as *M. incognita* and confirmed by isozyme gel electrophoresis (Phast System, Pharmacia). Molecular characterisation was performed by Ye *et al.* (2015) on root-knot nematode populations from turfgrasses samples by DNA sequencing on the ribosomal DNA 18S, ITS and 28S D2/D3. Species-specific primers were developed to identify turfgrass root-knot nematode through simplex or duplex PCR. Root knot nematode attack was observed in thippali at Vellanikkara (Plate A), but the species had not been identified. In this juncture, the study was undertaken with the objective to identify the species of root knot nematode infesting thippali.

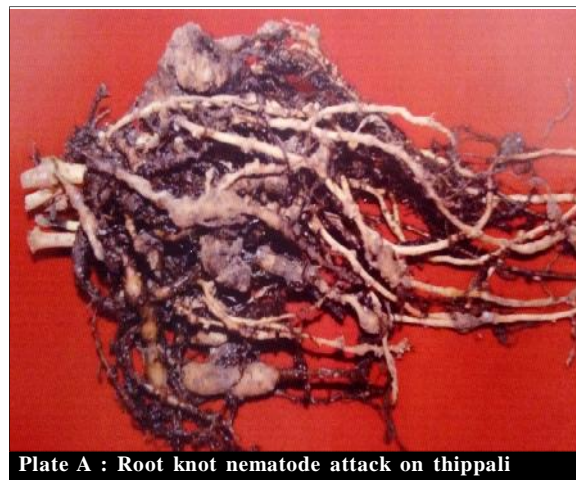


Plate A : Root knot nematode attack on thippali

MATERIAL AND METHODS

The study was carried out in Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, Kerala.

Preparation of denematized potting mixture :

Sieved field soil, sand and well decomposed farm yard manure was mixed in the ratio 1:1:1 and this potting mixture was filled in earthen pots of size 25 cm. Formalin was poured into each pot in the ratio 1:20 and were

covered with polythene sheets and tied firmly. After two weeks, the polythene sheets were removed and the mixture in each pot was raked well. Soil samples were taken from each pot to test the presence of nematodes. These pots with denematized potting mixture were used for further pot culture studies.

Maintenance of pure culture of root knot nematode infesting thippali :

The rooted cutting of thippali, variety Viswam were planted in pots filled with denematized mixture for the maintenance of nematode culture. Earthen pots of size 25 cm diameter with denematized potting mixture were used for raising potted plants. Two number of thippali cuttings with three nodes each were planted in each pot. Plants were irrigated periodically to maintain the wet condition of soil. Pureculture of the nematode was maintained from single egg mass collected from infested thippali roots from College of Horticulture, Vellanikkara. The second stage juveniles of the nematodes that hatched from the egg mass were inoculated to the potted plants. Repotting and inoculation were repeated periodically for maintaining the pure culture of root knot nematode for different experiments.

Identification of nematode :

The character most frequently used for *Meloidogyne* species identification is the morphology of perineal pattern, which is located in the posteriorbody region of adult females. This area comprises the vulva – anus area (perineum), tail terminus, striae. The species of root knot nematodes were identified by the perineal pattern of white females collected from root galls.

Collection of white females by staining technique :

White females were collected by cotton blue-lactophenol staining technique (Franklin and Goodey, 1949). Root samples from the culture plants were washed in stream of tap water to remove soil particles and the root knots were separated. It was then wrapped in muslin cloth. This small bag with root galls were plunged into boiling lactophenol containing 0.1 per cent cotton blue till the root tissues becomes soft. The root knots were removed from muslin cloth and were kept in Petri plate and washed in water to remove excess strain. Then it was transferred to a microscope slide containing a drop of lactophenol and dissected with the help of microscope.

The white females, which were stained light blue, came out of the root knots in large numbers and were collected and transferred to a glass vial containing lactophenol (Plate B). The species was then identified from the Department of Nematology, IARI, New Delhi.



Plate B : White Female of *Meloidogynearenaria* (40x)

RESULTS AND DISCUSSION

The species of root knot nematode was identified as *Meloidogyne arenaria* from the Department of Nematology, Indian Agricultural Research Institute, New Delhi and is commonly known as groundnut root knot nematode. It is a polyphagous nematode, attacking both monocotyledons and dicotyledons. The infestation of *M. arenaria* in thippali, is reported for the first time in India in this study.

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