Biological Forum – An International Journal **16(6): 15-17(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Isolation, Identification and Characterisation of Root-knot Nematode in Mulberry (*Morus alba* L.)

***Bharath K.B.*[1*], *Vinoda K.S.*[2], *Kavitha T.R.*[3], *Banuprakash K.G.*[4] *and Shashidhar K.R.*[5]**

[1]*Research Scholar, Department of Sericulture, UAS(B), GKVK, Bengaluru (Karnataka), India.*
[2]*Associate Professor, Department of Sericulture, UAS(B), GKVK, Bengaluru (Karnataka), India.*
[3]*Assistant Nematologist &Professor, AICRP (Nematodes), Department of Plant Pathology, UAS(B), GKVK, Bengaluru (Karnataka), India.*
[4]*Associate Professor, Department of Sericulture, UAS (B), GKVK, Bengaluru (Karnataka), India.*
[5]*Scientist (Sericulture), ICAR-KVK, UHS-Bagalkot, Tamaka, Kolar (Karnataka), India.*

(Corresponding author: Bharath K.B.)*
(Received: 27 March 2024; Revised: 15 April 2024; Accepted: 03 May 2024; Published: 15 June 2024)
(Published by Research Trend)

ABSTRACT: The rhizosphere of mulberry (*Morus alba* L.) is a crucial zone of microbial activity, where root-knot nematodes (RKN), particularly *Meloidogyne* sp., pose severe agronomic challenges. This study aimed to isolate and characterize RKN species infesting mulberry roots in Kalyapura Village, Karnataka, India. The research was conducted in a six-year-old mulberry plantation (variety V1) with red sandy loam soil, following standard agronomic practices. Infested root samples were processed using the root incubation method and nematodes were examined under a stereo-zoom binocular microscope. Detailed morphological analysis, focusing on perineal patterns, identified the predominant species as *Meloidogyne incognita*. The identification was corroborated by comparison with established morphological descriptions. This infestation is associated with significant agronomic impacts, including stunted plant growth, leaf chlorosis, and root galls, leading to compromised water and nutrient uptake. Consequently, the quality and yield of mulberry leaves, essential for the sericulture industry, are adversely affected. The study underscores the necessity for integrated nematode management strategies to mitigate the detrimental effects of RKN on mulberry cultivation and ensure the sustainability of silk production. These findings contribute to the broader understanding of RKN dynamics in mulberry ecosystems and the development of targeted control measures.

Keywords: Mulberry, Root-knot nematode, Root incubation, Perineal pattern, *Meloidogyne incognita.*

INTRODUCTION

The rhizosphere soil is more intensified zone of microbial activity where the population of microorganisms is comparatively high (Katznelson, 1965). The association of microorganisms with the plant root system may be mostly beneficial and sometimes become detrimental. Among the rhizosphere organisms of mulberry, fungi and nematodes contribute to a markedly higher population (Nandi *et al.*, 2004). In the global front, forty-two nematode species belonging to 24 genera were reported to be associated with mulberry as a pest (Swamy and Govindu, 1965). Among them, nematodes species belonging to genera *Meloidogyne*, *Rotylenchulus*, *Helicotylenchus*, *Hoplolaimus* and *Xiphinema* were majorly reported from India.

The root-knot nematode (RKN) are parasites of underground roots, which is difficult to recognize and hence the damage symptoms very often go unnoticed (Sengupta and Govindaiah 1991). The affected plants show stunted growth, marginal necrosis and yellowing of leaves. The characteristic knots or galls appear on the roots and affect the utilization of water and nutrients resulting in poor plant growth (Govindaiah *et al.*, 1991). Arunakumar *et al.* (2018) delineated that *Meloidogyne incognita* infesting mulberry caused 20-50 per cent leaf yield loss apart from deteriorated leaf quality. This connotes the importance of characterising the nematode species appertaining to mulberry ecosystem.

In this purview, an experiment was conducted to isolate and characterise the RKN associated with mulberry roots.

MATERIAL AND METHODS

A. Experiment site details

The experiment was carried out in the RKN infested mulberry garden in Kalyapura Village, Shidlaghatta Taluk, Chikkaballapura District *i.e.*, in the Eastern Dry Zone (Zone-5) of Karnataka, India at 13°14'20"N latitude and 77°52'17"E longitude, at an altitude of 904m above mean sea level.

The mulberry plantation selected for isolation of nematodes had red sandy loam type of soil with six years old V1 variety plants, planted at the spacing of 90

× 90cm; bottom pruning (Kolar method) was followed; field was irrigated in two-days interval with drip irrigation facility *i.e.*, based on soil moisture conditions; organic manures and inorganic fertilizers were applied in accordance with the recommended package of practice. Except for the management of RKN, the selected field was well maintained.

B. Isolation and identification of mulberry RKN

To isolate the RKN, five grams of infested root sample was processed following the root incubation method (Ayoub, 1977). After incubation for 48 hours, the suspension column was made up to 200 ml, out of which 10 ml was pipetted out and used for the isolation of root-knot nematode infesting the mulberry plants in the experimental site. The severely infested plants were tagged based on the above ground visual symptoms before collecting the root samples (Fig. 1).

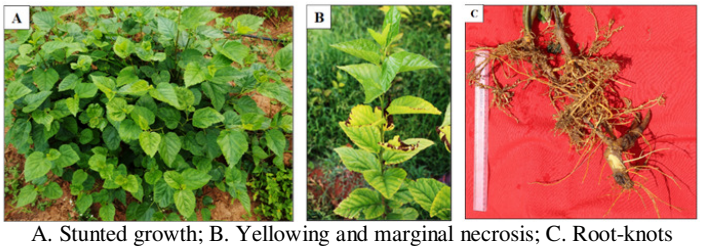
The RKN infested roots were washed free of soil. Female nematodes were isolated from the well-developed galls under a stereo-zoom binocular microscope and transferred to a petri plate containing water. The posterior portion was cut with a perineal pattern knife (Taylor and Sasser 1978) and the body contents were cleared. The cleaned posterior portion of the female was further trimmed and transferred to a drop of glycerin on a clean microscopic glass slide. Bits of glass wool were placed around the trimmed portion to avoid damage. A cover slip was placed over the trimmed portion and sealed, then observed under the stereo binocular microscope. The species identification and characterisation were done based on the perineal patterns as described by Chitwood (1949).

C. Taxonomical status of RKN

Phylum	Nematoda
Class	Secernentea
Sub class	Diplogasteria
Order	Tylenchida
Sub order	Tylenchina
Super family	Tylenchoidea
Family	Heteroderidae (Meloidogynidae)
Sub family	Meloidogyninae
Genera	*Meloidogyne*
Species	*incognita, javanica, arenaria, hapla*

D. Differentiation between the RKN species based on the perennial pattern

Characters	***Meloidogyne incognita***	***Meloidogyne javanica***	***Meloidogyne arenaria***	***Meloidogyne hapla***
Striae	Striae are smooth, wavy and sometimes in a zig-zag pattern	Striae are smooth and somewhat wavy	Striae are smooth and slightly wavy, often extended laterally, forming wings on one or both lateral sides of the patterns	Striae are close, smooth and wavy, some patterns form wings on one or both lateral side
Lateral lines and ridges	Lateral lines are absent	Unique distinct lateral ridges across the pattern and fade away around the tail terminus	Distinctive lateral ridges are absent and the pattern is forked, irregular lateral fields	Lateral ridges are absent, but the lateral fields are marked by irregularities in the striae
Dorsal arch	Squarish high dorsal arch containing a distinct whorl around the tail terminus	Dorsal arch is often low and rounded, frequently possessing a whorl in the tail terminus area	Dorsal arch is low and intended near the lateral fields, forming rounded shoulders	Dorsal arch is usually low and rounded and the perennial pattern is stippled with subcuticular markings



A. Stunted growth; B. Yellowing and marginal necrosis; C. Root-knots

Fig. 1. Symptoms of RKN infestation in mulberry.

RESULTS AND DISCUSSION

The RKN juveniles isolated from the root samples collected from the experimental site possessed the following characters – body was typically saccate and spheroid like with a distinct neck; stylet was found to be slender with strong basal knobs; oesophageal median bulb was observed and the excretory pore was found to be situated between stylet knob and the median bulb; perineal patterns were found surrounding the anus and vulva similar to that of human fingerprint pattern, at the side exactly opposite to the neck; perineal pattern striae were smooth and wavy without any lateral lines, and the dorsal arch was high having a distinct whorl around the tail terminus (Fig. 2). The key observations on perineal pattern using scanning electron microscope images were in line to those described for *Meloidogyne incognita* by Chitwood (1949). The described morphological characteristics confirmed that the nematode species associated with the mulberry plants was *M. incognita* (Kofoid and White).

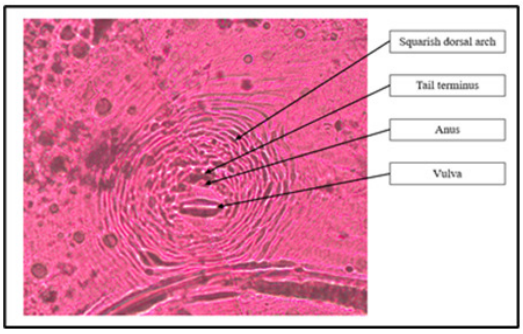


Fig. 2. SEM image of the perineal pattern in Meloidogyne incognita female larva.

The morphological characteristics observed were in consonance with that of those described by Narasimhamurthy (2010). Nalini Singh and Sunita Chahar (2021) reported on morphological description of *M. incognita* infesting cucumber roots which were also on similar lines. The perennial patterns of female juveniles, morphological characteristics and the DNA-specific markers confirmed the pathogenicity of nematode species, *M. enterolobii* in the mulberry trees in Hainan Province of China (Sun *et al.,* 2019), *M. arenaria* on white mulberry in Europe (Castillo *et al.*, 2001) and *M. enterolobii* in *Morus nigra* L. seedlings (Paes-Takahashi *et al.*, 2015). Stella *et al.* (2022) revealed that the species of root-knot nematode associated with papaya plants were *M. javanica* based on the perineal pattern of female nematodes, specifically the lateral lines.

CONCLUSIONS

The morphological studies and the perineal patterns of the nematode harbouring in mulberry confirmed that the major species involved in causing the root-knots is *Meloidogyne incognita* ((Kofoid and White) Chitwood. The study proves to be significant because the infestation of RKNs not only affects the growth and development of the crop but also affects the physiology ultimately resulting in inferior quality of foliage. Further, since mulberry leaf is the sole source of nutrition for silkworm, *Bombyx mori* L., the cocoon crop and quality of rawsilk are severely affected causing huge economic loss to the farmer.

FUTURE SCOPE

A roving survey may be conducted in different zones of Karnataka practising sericulture, to study the diversity of RKN infesting mulberry. Molecular characterisation could also be emphasized along with morphological characterisation.

Acknowledgement. The authors are thankful to AICRP (Nematodes) Laboratory, UAS-Bangalore, GKVK for providing required laboratory tools and assistance. They are gratified by the farmer's cooperation in conducting the experiment.

Conflict of Interest. None.

REFERENCES

Arunakumar, G. S., Revanna, S., Kumar, V., Yadav, V. K. and Sivaprasad, V. (2018). Studies on scanning electron microscopy and fungal association with root-knot nematode in major mulberry growing areas of Southern Karnataka. *Journal of Entomologyand Zoological Studies*, *6*(4), 511-518.

Ayoub, S. M. (1977). Plant Nematology – An Agricultural Training Aid. *Department of Food and Agriculture, Sacramento, California, USA*, 156.

Castillo, P., Vito, M., Di Vovlas, N. and Jimenez-Diaz, R. M. (2001). Host-parasite relationships in root-knot disease of white mulberry. *Plant Diseases, 85*, 277–281.

Chitwood, B. G. (1949). Root-knot nematode-I.A. Revision of the genus *Meloidogyne Goildi*, 1887. *Proceedings of the Helminthological Society, Washington*, 92- 104.

Govindaiah, Dandin, S. B. and Sharma, D. D. (1991). Pathogenicity and avoidable leaf yield loss due to *Meloidogyne* in mulberry (*Morus alba* L.). *Indian Journal of Nematology*, *21*(1), 96-99.

Katznelson (1965). Nature and importance of rhizosphere. *International Symposium on Ecology of Soil Borne Plant Pathogens*, 187-209.

Nalini Singh and Sunita Chahar (2021). Isolation and morphological identification of root knot nematode from cucumber roots. *International Journal of Botany Studies, 6*(5), 547-550.

Nandi, S., Das, P. K., Katiyar and Rajanna(2004). Study on rhizosphere microflora of mulberry. *Indian Journal of Sericulture*, *43*(2), 213-215.

Narasimhamurthy, T. N. (2010). Evaluation of indigenous and commercial bioagents for management of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood 1949 in mulberry (*Morus alba* L.). *Ph.D. Thesis, University of Agricultural Sciences, Bangalore, India*, 122.

Paes-Takahashi, V., Soares, P. L. M., Carneiro, F. A., Ferreira, R. J., Almeida, E. J. and Santos, J. M. (2015). Detection of *Meloidogyne enterolobii* in mulberry seedlings (*Morus nigra* L). *Ciencia Rural*, *45*(5), 757-760.

Sengupta, K. and Govindaiah, K. K. (1991). Diseases and pests of mulberry and their control. *Central Sericulture Research and Training Institute Mysore, India*, 1-20.

Stella Nostra Gracia Plena, Siwi Indarti, Ani Widiastuti and Nugroho Susetyo Putra (2022). Identification of the root-knot nematode species associated with *Carica papaya*. *Biodiversitas, 23*(12), 6212-6217.

Sun, Y. F., Long, H. B. and Lu, F. P. (2019). First report of the root-knot nematode *Meloidogyne enterolobii* infecting mulberry in China. *Plant Diseases*, *103*(9), 2481-2481.

Swamy, B. C. N. and Govindu, H. C. (1965). A preliminary note on the plant parasitic nematodes of the Mysore state. *Indian Phytopathology*, *19*, 233-240.

Taylor, A. L. and Sasser, J. N. (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). *International Meloidogyne Project North Carolina State University*, 101-105.

How to cite this article: Bharath K.B., Vinoda K.S., Kavitha T.R., Banuprakash K.G. and Shashidhar K.R. (2024). Isolation, Identification and Characterisation of Root-knot Nematode in Mulberry (*Morus alba* L.)**.** *Biological Forum – An International Journal, 16*(6): 15-17.