Annual Research & Review in Biology

Volume 39, Issue 9, Page 79-86, 2024; Article no.ARRB.122438
ISSN: 2347-565X, NLM ID: 101632869
(Past name: Annual Review & Research in Biology, Past ISSN: 2231-4776)

Nematode Diversity and Root-Knot Nematode Management Approaches for Sustainable Mulberry Production

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/arrb/2024/v39i92121

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122438

Review Article

Received: 17/06/2024
Accepted: 22/08/2024
Published: 26/08/2024

ABSTRACT

Mulberry ecosystems are beset by the clandestine menace of nematodes, precipitating devastating consequences on plant productivity. This review highlights the importance of nematode diversity in mulberry ecosystems and explores various eco-friendly and sustainable approaches for managing the root-knot nematode, *Meloidogyne incognita* involving cultural, biological and host resistance methods. Various eco-friendly management strategies *viz.*, cultural approaches like summer deep ploughing, mulching and trap crops; biological methods like bio-agents and plant extracts; and resistant varieties against the root-knot nematode infecting mulberry are reviewed and discussed providing an impetus for integrating multiple approaches for effective and sustainable root-knot nematode management.

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***Cite as:** KB, Bharath, Vinoda KS, Kavitha TR, and Banuprakash KG. 2024. "Nematode Diversity and Root-Knot Nematode Management Approaches for Sustainable Mulberry Production". Annual Research & Review in Biology 39 (9):79-86. https://doi.org/10.9734/arrb/2024/v39i92121.*

Keywords: Mulberry; nematode diversity; Meloidogyne incognita; cultural; biological approaches; host resistance.

1. INTRODUCTION

Mulberry (*Morus alba* L.), a highly adaptable perennial plant, thrives in diverse climatic conditions, from temperate to tropical regions, and tolerates various soil types. As the sole food source for silkworms, mulberry foliage plays a crucial role in determining the quality of raw silk. The quantity and quality of leaves produced per unit area directly impact cocoon production and raw silk quality, making foliage the primary economic component of mulberry cultivation.

Mulberry leaf is a crucial factor in obtaining a successful cocoon crop, contributing approximately 38.20% to its success. As the primary source of nutrition for silkworms, mulberry leaf plays a vital role in cocoon production. However, its fast-growing, perennial, and lush green characteristics make it susceptible to diverse biotic stresses, including diseases (pathogens) and pest (insect and non-insect) damage, which can degrade its nutritive values [1]. A markedly higher population of nematodes was observed in the rhizosphere soil of mulberry [2].

Nematodes comprise the phylum Nematoda, which includes parasites of plants, animals, humans, and species that parasitize bacteria, fungi, algae, and other nematodes. Plant parasitic nematodes are thread-like, pseudocoelomate, transparent, triploblastic and bilaterally symmetrical invertebrates. Four out of every five multicellular animals on the planet are nematodes [3]. Cobb [4] calculated that if the nematodes resident in a single acre of soil near San Antonio, Texas, USA, were to proceed in a head-to-tail procession to Washington DC, some 2000 miles away, the first nematode would reach Washington before the rear of the procession left San Antonio. Several plant-parasitic nematodes belonging to different genera have been reported in the rhizosphere soil of mulberry gardens. The nematodes readily perpetuate and cause stunted growth, yellowing of leaves, wilting and overall general growth decline.

2. DIVERSITY OF NEMATODES IN THE MULBERRY ECOSYSTEM

Nematodes, with more than 14000 described species, are distributed in almost every habitat on Earth and represent more than 80% of metazoan taxonomic and functional diversity in soil [5]. In Surtsey, the number of nematode genera has steadily increased from one taxon in 1970 to 25 taxa in 2012 [6]. The nematode disease incidence was found to be higher in irrigated mulberry gardens and comparatively unnoticed in rainfed gardens [7].

Among the nematodes, forty-two species belonging to 24 genera were associated with mulberry as reported from almost all the countries, where sericulture is being practiced [8]. Of these 24, the nematodes belonging to five genera *viz.*, *Meloidogyne*, *Rotylenchulus*, *Helicotylenchus*, *Hoplolaimus* and *Xiphinema* were reported from India. A survey conducted by Hirata [9] in Gumma Province, Japan, reported eight genera of nematodes *viz.*, *Meloidogyne*, *Criconema*, *Pratylenchus*, *Xiphinema*, *Trichodorus*, *Longidorus* and *Helicotylenchus* associated with mulberry. Of these, *Pratylenchus* and *Criconema* were dominant while *Meloidogyne* and *Longidorus* were the most frequently encountered nematodes in the alluvial soils. The studies conducted in Kumamoto province of Japan revealed the presence of nematodes belonging to the genera *Meloidogyne*, *Paratylenchus*, *Helicotylenchus* and *Xiphinema* that were distributed at a depth ranging from 10 to 100 cm of soil [10]. About 16 nematode species belonging to *Meloidogyne*, *Rotylenchulus*, *Pratylenchus*, *Longidorus* and *Aphelenchoides* genera were studied in the plant stocks of mulberry nurseries in Uzbekistan [11]. Ramakrishnan and Senthikumar [12] reviewed and documented about 48 species of nematodes belonging to 24 genera that are associated with the mulberry in different mulberry growing regions of the world. About 89 species of nematodes under 34 genera, 21 subfamilies, 17 families, 12 superfamilies and 6 suborders belonging to 4 orders, were described and reported so far from India [13].

A few major nematodes in the mulberry ecosystem include dagger nematode (*Xiphinema* sp.), spiral nematode (*Helicotylenchus* spp.), *Scutellonema* sp. and root-knot nematode (RKN) (*Meloidogyne incognita*). The dagger nematode (*Xiphinema basiri*) in mulberry was found to be dominant in 10 – 15 cm depth of soil. On infestation, it fed the root tips up to the vascular elements of young roots, but up to cortical parenchyma in older roots. The feeding duration was long, inducing reduced root system, lesions, discolouration, disintegration and decay of cortical tissue, terminal and sub-terminal swelling, forming curly tip or fish hook leading to stunted or declined growth of mulberry plants [14]. The spiral nematode (*Helicotylenchus indicus*) in mulberry ecosystems is generally found with its head end embedded and feeding on roots, also known to cause secondary infection generally around their feeding sites. The characteristic symptoms include reduced length and weight of the shoot resulting in the reduced number of leaf buds and leaves [15]. The *Scutellonema* spp. infecting mulberry feed on the peripheral tissues causing necrosis which further leads to secondary infection by other fungi and bacteria [16]. *M. incognita* infested plants show stunted growth, marginal necrosis and yellowing of the leaves. The characteristic knots or galls appear on the roots and affect the utilization of water and nutrients resulting in poor plant growth [17].

The severity of attack and damage by the nematode depends on the soil and climatic conditions of the different areas. The nematode disease incidence was very high in red sandy soil, followed by red loamy soil and the lowest incidence was noticed in black cotton soil. Among different farming systems, irrigated gardens had high incidence and intensity of nematodes, and they went unnoticed under rainfed conditions [12]. Bina and Mohilal [18] conducted a study on the population behaviour of plant parasitic nematodes associated with mulberry plants in the farms of Imphal West, Bishnupur, Thoubal and Imphal East districts of Manipur, India. The variation in the nematode population in the selected mulberry gardens of four districts may be due to topographical variations, variations in rainfall, humidity and cultivation practices accompanied by ploughing frequency and application of fertilizers. The correlation of soil parameters with the abundantly found nematode, *Helicotylenchus graminophilus* indicated that soil parameters like organic carbon, nitrogen, phosphorous and soil p^H had a positive correlation, while potassium had a negative correlation with the abundance of the nematode population, potassium accumulates in the infestation site of the root and inhibits the metabolic activity of the nematodes.

2.1 Root-knot Nematode in Mulberry

The RKN, *M. incognita* is a significant economic pest, causing substantial damage to plant growth and yield worldwide. Estimated annual losses attributed to *M. incognita* alone reach $100 billion [19]. In severe cases, high RKN damage can result in total crop failure [20]. *M. incognita* alone is known to cause 20-50 percent loss in mulberry leaf yield apart from deteriorated quality [21].

M. incognita has three life stages - egg, larva with four juvenile stages (J_1, J_2, J_3, J_4) and adult of which, the J_2 infects the host plants [22]. The female larva enters the roots, harbours in the sub-epidermal layer and starts feeding on the parenchymatous cells causing hypertrophy and hyperplasia that induce characteristic knots. The larvae undergo four moults in the roots and develop into mature oval or spherical egg-laying females. Each female lays 200-300 ellipsoidal eggs covered with a gelatinous substance. The eggs hatch and larvae are liberated into the soil under favourable conditions. The life cycle is completed in 30-40 days and 2-3 such cycles are noticed per annum. A temperature of 15-30˚C and soil moisture of 40-60 per cent are more favourable for the growth and development of RKNs [23]. The size and number of galls induced by RKNs keep on increasing as the generations are repeated in the root tissues. Apart from damaging the parenchyma tissue, the cracks and holes in the galls invite secondary root infections. Thus, the root-knot infected plants show symptoms of both nutrient deficiency and other root diseases like root rot [24].

3. MANAGEMENT OF ROOT-KNOT NEMATODES IN MULBERRY ECOSYSTEM

During infection, nematodes establish a close relationship with their host, inducing significant cellular changes. The formation of giant cells (GC) is a hallmark of RKN infection, characterized by repeated nuclear division without cell division, resulting in a multinucleate cell [25]. Initially, a second nucleus appears, indicating interrupted cell development, leading to a cell with two nuclei due to disturbed cell plate formation. Nuclear division continues, resulting in GC containing up to 100 nuclei, with sizes reaching up to 400 times those of regular root vascular cells [26]. RKN continuously extracts cytoplasm from the infected cell, creating a metabolic sink. Nearby cell division and GC formation lead to root-knot formation. In response to RKN damage, the host activates defense mechanisms, including wound-responsive jasmonate and ethylene pathways [27]. However, a strong decrease in defenserelated hormone pathways, primarily ethylene and salicylate, is observed in GC, developing galls, and surrounding tissues.

Vijaya Kumari and Sujathamma [28] reported that *M. incognita* infestation led to a significant per cent reduction in the leaf yield (kg/ha/yr) in all the promising cultivars of mulberry *viz.*, Tr-10 (23.14%), DD (25.14%), V-1 (26.36%), RFS-175 (27.66%), K-2 (28.52%) and MS-8 (30.58%) which highlights the significance of managing nematodes in the mulberry ecosystem**.** Effective management of RKN is crucial and relies heavily on two key factors: the initial nematode population and the host plant. Since complete control or eradication of RKN is unfeasible, various management techniques have been explored and recommended. These include cultural, physical, chemical, biological, host plant resistance and integrated management approaches. The use of chemical nematicides for nematode management has been found to result in toxic mulberry leaves, which can be harmful to silkworms. Additionally, chemical nematicides can have deleterious effects on soil health. Consequently, there is a growing interest in eco-friendly and non-chemical approaches for managing root-knot nematodes in mulberry. This review focuses on three promising alternatives: cultural methods, biological methods and host plant resistance. These environmentally friendly strategies offer a more sustainable solution for managing root-knot nematodes in mulberry.

3.1 Cultural Interventions

Cultural approaches have been shown to effectively mitigate plant diseases, including RKN infestations in mulberry gardens. Deep ploughing up to a depth of 30-40 cm during the summer season can increase soil temperature, destroying heat-sensitive eggs and juveniles of the nematode [29]. Soil solarization, a technique involving the covering of soil with a plastic film during the summer season, effectively increases soil temperature, leading to the destruction of nematode egg masses and populations [30]. The higher temperature and lower humidity developed in the soil due to summer deep ploughing could kill the nematode eggs and juveniles present in the soil.

Mulching with green leaves increases the organic matter and favours the antagonistic activity against soil-borne pathogens like nematodes [31]. Mulching with neem leaves has been found to reduce root-knot disease and prevent leaf yield loss [32]. Pongamia leaves have also been shown to reduce RKN infestation when used as a mulching material [33]. Furthermore, using neem and pongamia cakes as mulches and organic manures reduced RKN infestation by affecting the number of root-knots, egg masses and juveniles of the nematode [32].

The use of trap crops is a well-established strategy for managing RKN. Intercropping *Tagetes patula* (marigold) with mulberry at 30 cm distance decreased RKN infection by 75.84 % without affecting the leaf yield and its impact is attributed to its root exudates α-terthienyl [34]. Intercropping *Amaranthus viridis* L. in mulberry gardens as a trap crop is effective as it is more susceptible to RKN than mulberry, it attracts and traps the nematode, thereby reducing the severity of RKN infestation in mulberry [35]. Cultivating cover crops like cowpea and sunnhemp in mulberry gardens can reduce RKN populations. The presence of cover crops physically prevents nematodes from migrating to other areas [36].

3.2 Biological Interventions

Biological approaches provide a sustainable alternative for managing plant pests and diseases, offering a unique combination of bioavailability, specificity and eco-friendliness [37]. By harnessing the power of bioagents, including parasites, predators, fungi, bacteria, viruses, and plant extracts, bio-pesticides deliver a targeted approach that prioritizes the safety of non-target species, beneficial insects, higher animals and humans, while minimizing environmental impact.

3.2.1 Bio-agents

Bio-control agents exhibiting anti-nematode activity were evaluated to assess their efficacy under field conditions. In pot culture experiments, soil application of *Trichoderma harzianum* THN1 strain significantly inhibited egg hatching, reduced root-knot formation, and enhanced leaf yield [38]. Similarly, soil application of *Trichoderma viride* (2×10 cfu/g) and *Pseudomonas fluorescens* (1×10 cfu/g) effectively suppressed the formation of galls, egg masses, and M. incognita population in mulberry, while also promoting growth and yield by increasing shoot length, leaf number and leaf yield [39]. The combined soil application of *P. fluorescens* + *T. viride* (each at 10 g/plant) was effective in checking the RKN disease and improving the growth and yield parameters of mulberry [40].

Application of sericompost, a nutrient-rich soil amendment made from silkworm litter and rearing waste, enriched with beneficial microbes such as phosphate solubilizing bacteria *Bacillus megaterium* and *Azotobacter chroococcum*, as well as antagonistic microbes *Trichoderma harzianum* and *Trichoderma pseudokoningii*, was found to effectively reduce root-knot disease in mulberry. Additionally, this treatment improved soil health, demonstrating the potential of bio-agent enriched sericompost as a sustainable and eco-friendly approach to managing soil-borne diseases [41]. The application of *T. viride* at 5 kg/ha with 5 tons FYM (farm-yard manure) markedly reduced the nematode population in both soil and roots, achieving a substantial reduction of 79.82% in soil nematodes and 85.21% in root nematodes at 120 days post-treatment [42]. Target-specific nematode population inhibitor, Nemahari showed a maximum per cent reduction in the egg masses and number of galls (86.45% and 85.30%, respectively) in V-1 mulberry [43]. Bionema (*Pochonia chlamydosporium*) also reduced the root-knot disease up to 80-85% [44].

3.2.2 Plant extracts

The plant extracts contain a wide array of secondary metabolites, such as alkaloids, saponins, flavonoids, tannins, terpenes, acetylenes and glucosinolates, which contribute to their antimicrobial activity [45]. Foliar extracts of various plant species have been shown to possess anti-nematode properties, effectively inhibiting egg hatching and inducing mortality in J2 juveniles of *M. incognita* [46].

The homeopathic medicines, *Cina* mother tincture (MT) and potentized *Cina 200C,* prepared from the flowering meristems of *Artemisia nilagirica*, were applied as foliar sprays. The treated plants showed a significant reduction in nematode infection in terms of root gall number and nematode population in the root and the plants exhibited improved growth in terms of fresh biomass of shoot and root, length of shoot and root, number of leaves, leaf surface area, root and leaf-protein content [46]. The neem seed kernel extract is also known to reduce the nematode population [47].

3.3 Host Plant Resistance

Utilizing host resistance is a primitive, efficient and eco-friendly principle in plant disease management. Developing resistant varieties is an effective approach to combat root-knot nematodes [48]. Resistant varieties hinder nematode entry and reproduction, thereby reducing disease incidence [49]. However, developing disease-resistant mulberry varieties poses a challenge due to its perennial woody nature, requiring many years of breeding and selection efforts.

Extensive screening of diverse mulberry germplasm, including both exotic and indigenous accessions, was conducted under field conditions to identify potential sources of resistance against *M. incognita*. Eight accessions, namely BR-8, Karanjtoli-1, Hosur-C8, Nagalur Estate, Tippu, Calabresa, Thai Pecah, and SRDC-3, were identified as promising genetic sources for resistant rootstocks [50]. Similarly, RFS 135, exhibited resistance to *M. incognita*, while C-20 and DD showed moderate resistance. Further investigation into the mechanism of resistance revealed a positive correlation between *M. incognita* resistance and peroxidase enzyme activity in mulberry genotypes [51].

An evaluation of 50 indigenous mulberry genotypes against *M. incognita* showed varying degrees of infestation. Notably, 12 genotypes - Jathuni, BerC-776, Kokuso-13, Nan-nayapathi, Muki, Kolitha-7, Bilidevalaya, V-1, Ace-199, Nagaland local and Sultanpur - demonstrated resistance against root-knot nematode infestation [52]. Similarly, screening six varieties RFS-175, K-2, MS-8, Tr-10, DD and V-1 against *M. incognita* revealed that Tr-10, DD and V-1 exhibited better tolerance, characterized by reduced root-knot incidence, and superior growth and development compared to RFS-175, K-2 and MS-8 [28].

4. CONCLUSION

This review provides a comprehensive overview of the significance of knowing the diversity of nematodes infesting mulberry and various means to manage the root-knot nematodes in mulberry ecosystems. The emphasis on the need for eco-friendly and sustainable management practices to mitigate root-knot nematode infestations involving cultural, biological and host resistance approaches is detailed here. Further, this could provide an impetus for exploring the diversity of nematodes and their interactions with mulberry genotypes which can lead to the identification of resistant varieties and the development of targeted management practices. It can also help in integrating multiple approaches for effective root-knot nematode management.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, *etc*.) have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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