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Eco-Friendly Management of Root-Knot Nematode in Mulberry - An Overview

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Authors Contribution

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Abstract

Mulberry (*Morus alba* L.) is the only host plant of silkworm (*Bombyx mori* L.) which is mainly grown for its leaf. The yield of mulberry leaf gets affected by various diseases and among them, root-knot caused by *Meloidogyne incognita* is one of the major diseases which affects both quantity and quality of leaf. Even though nematicides were reported to provide better control under field conditions, due to their deleterious effect on soil health and possible toxicity to silkworms, eco-friendly approaches to mitigate the root-knot nematode have received greater attention. Various eco-friendly management strategies *viz.,* resistant and tolerant varieties; cultural methods like summer ploughing, soil solarization, trap crops; biological methods like botanicals and biocontrol agents against root-knot nematode infecting mulberry are reviewed and discussed.

Keywords : Mulberry, *Meloidogyne incognita*, Cultural and biological control

Mulberry (*Morus alba* L.) is a perennial crop, mainly grown for its leaves which is the sole food source for silkworm (*Bombyx mori* L.). The quality and quantity of mulberry leaf decide the cocoon production and in turn quality of silk. However, mulberry leaf yield and quality gets affected by various biotic and abiotic factors. Among biotic factors, root-knot caused by *Meloidogyne incognita* (Kofoid and White) Chitwood is the one affecting more than 80 per cent of mulberry plantations in various mulberry growing regions (Teotia and Sen, 1994). In India, it was first reported from Mysore in 1966 (Narayanan *et al*., 1966). The loss due to root-knot nematode is estimated upto 10 per cent in addition reduction in the nutritive value of mulberry leaves (Sharma, 1999 and Nelaballe, 2013). Feeding the silkworm with unhealthy leaves affects their growth, development, as well as cocoon yield and quality (Philip *et al*., 1994 and Gupta, 2001). Apart from directly affecting the mulberry leaf production, infection of root-knot nematode also weakens the plants which in turn facilitates easy entry of secondary invading pathogens (Powell, 1971 and Webster, 1985).

There are six major species of *Meloidogyne* causing root-knot in mulberry (*M. incognita, M. javanica, M. hapla, M. arenaria, M. arenaritharnsi* and *M. mali*), among them *M. incognita* was reported to be more prevalent (Narayanan *et al.,* 1966; Todia, 1984 and Ertian, 2003). *M. incognita* is reported to have a wide host range of nearly or more than 2000 host plants (Tiwari *et al*., 2009) and produces egg masses that survive well under unfavorable conditions (Moens *et al*., 2009) which made difficult to reduce the inoculum level in infected soil.

Various management strategies *viz.,* cultural, physical, biological and chemical methods (Chitwood, 2002) were followed in mulberry to mitigate root-knot nematode infestation. Among them, chemical methods through the application of nematicides were known to have better efficacy in field conditions (Giacometti *et al*. 2010 and d'Errico *et al*. 2011). But these soil-fumigants and nematicides used for the management of plant-parasitic nematodes were reported to have a deleterious effect on soil health, human health, groundwater contamination and also includes possible toxicity to silkworms (Chitwood, 2003 and Govindaiah *et al*., 2003). Hence, restrictions were imposed on the usage of those chemical compounds in agriculture to reduce the possible deleterious effects. Restrictions on the use of chemicals have limited the availability of management strategies against plant-parasitic nematodes making to think of eco-friendly approaches for the management of root-knot nematode keeping in the view of both mulberry and silkworm. The present review focussed on compilng the various eco-friendly approaches used for the management of root-knot nematode which helps to overcome the deleterious effect of nematicides on soil health.

1. Biology and Life Cycle

Root-knot nematode belongs to the order *Tylenchida*, family *Heteroderidae,* genus *Meloidogyne*. The nematode *M. incognita* is most commonly found to be associated with root-knot in various parts of India. It is a sedentary endoparasite where, nematodes become immobile by establishing a permanent feeding site (Wyss and Grundler, 1992). Further, it produces knots on the functional roots that affect the nutrient and water uptake. During the unfavorable conditions nematode gets to survive in the form of egg masses and root galls in the soil which serves as an inoculum for the next crop season (Goodell and Ferris, 1989). In *Meloidogyne* spp, a male nematode is vermiform and motile, while a female nematode is pear-shaped and sedentary. Females lay eggs in a gelatinous mass secreted by rectal glands, which protects the eggs from extreme environmental conditions (Moens *et al*., 2009). The first juvenile stage completes in the egg itself, the egg is then converted into the second juvenile stage (J_2) which is said to be an infective stage (Khalil, 2017). The life cycle of *Meloidogyne* spp depends on the prevailing temperature conditions, in general, *M. incognita* completes its life cycle from egg to egg within 25 days at 27ºC (Tiwari *et al*., 2009).

2. Infection Process and Symptomatology

Root-knot nematodes (*Meloidogyne* spp.) are biotrophic plant parasites (Abad *et al*., 2003) where the infection process involves initial puncture of a cell by J_2 juvenile through stylet and further invasion through secretions from stylet. The secretions contain proteins that are responsible for the development of a parasitic relationship with the host. The infective stage is J_2 juvenile, which enters the host tissue intercellularly, further establishes the feeding site and upon secretions stimulates the root cells to form the giant cell (Ralmi *et al*., 2016).

Upon infection and colonization by J_2 stage of *M. incognita* in mulberry plants exhibits chlorosis of leaves (initially older leaves), stunted growth of plants as foliar symptoms and below ground, it forms galls on the roots with poor root growth. The infestation also leads to poor growth, reduced quality and quantity of leaf as well as predispose the plants to secondary infection by other pathogenic microbes (Anamika and Sobita, 2012).

3. Eco-friendly Management Approaches

The chemical-based nematode management results in toxic mulberry leaves, which in turn, lead to the toxicity of silkworms. Because of this toxic effect on silkworms and various deleterious effects on soil health by nematicides, an eco-friendly and non-chemical approach for the management of root-knot nematode in mulberry received greater attention. The host resistance, cultural and biological approaches having great impetus in the management of root-knot nematode in mulberry were described below.

3.1 Host Resistance

Among the plant disease management principles, employing host resistance is one of the most primitive, efficient and eco-friendly methods. Developing resistant varieties is one of the efficient approaches to combat root-knot nematode (Ferraz and Mendes, 1992). These resistant varieties restrict the entry and reproduction of nematodes thereby reducing the disease incidence (Jenkins and Sasser, 1960). Since mulberry is a perennial woody plant it takes many years to develop a disease resistant varieties. Concerning host resistance in mulberry, resistant sources and varieties against *M. incognita* are described below.

Various exotic and indigenous germplasm of mulberry were screened against *M. incognita* under field conditions to identify the potential resistant sources. Among them, eight germplasm accessions *viz.,* BR-8, Karanjtoli-1, Hosur-C8, Nagalur Estate, Tippu, Calabresa, Thai Pecah and SRDC-3 were identified as potential genetic sources as resistant rootstock against root-knot nematode infecting mulberry (Arunkumar *et al.*, 2021). Similarly, out of 10 mulberry varieties/genotypes screened against *M. incognita* in a field experiment, only one variety RFS 135 has shown resistance reaction while C20 and DD varieties showed moderate resistance reaction. Further, deciphering the mechanism of action of resistant varieties against nematode showed a positive correlation between the *M. incognita* resistance and peroxidase enzyme activity in mulberry genotypes (Gnanaprakash *et al.,* 2016).

Screening six varieties *viz.,* RFS175, K2, MS8, Tr10, DD and V1 of mulberry against *M. incognita* revealed that Tr10, DD and V1 varieties have better tolerance compared to RFS175, K2 and MS8 varieties with reduced root-knot incidence and also showed better growth and development (Kumari and Sujathamma, 2016). Similarly, 50 indigenous genotypes of mulberry evaluated against *M. incognita* showed a varying degree of infestation by root-knot nematode in different genotypes. Among them, Jathuni, BerC-776, Kokuso-13, Nan-nayapathi, Muki, Kolitha-7, Bilidevalaya, Victory-1, Ace-199 (Panchagani), Nagaland local and Sultanpur showed resistance against root-knot nematode infecting mulberry (Padma, 2012).

3.2 Cultural Approaches

Mitigating plant diseases through the manipulation of different agronomic practices carried out during crop cultivation has remarkable output in reducing the plant disease severity and its source of inoculum in the field. Some of the cultural approaches were reported to be effective to reduce the root-knot nematode infestation in the mulberry garden.

Deep ploughing or digging to a depth upto 30-40 cm during the summer season leads to increased soil temperature. Since the eggs and juveniles of root-knot nematode are heat sensitive, they get destroyed due to the higher temperature in the soil upon summer ploughing (Sharma *et al.*, 1999). Further, mulching around the mulberry plants with neem leaves (1 t/ha) has reduced the root-knot disease and prevented leaf yield loss (Govindaiah *et al.,* 1997a). Similarly, pongamia leaves are also known to reduce the root-knot infestation in mulberry when applied as mulching material (Govindiah *et al.*, 1989 and Govindaiah *et al.,* 1997a). Apart from leaves, neem and pongamia cakes used as mulches and organic manures in the mulberry garden have reduced the root-knot infestation by affecting the number of root-knots, egg masses and juveniles of root-knot nematode (Govindiah, 1997b).

The utilization of trap crops for the management of different soil-borne plant pathogens is well documented. Among them, intercropping amaranthus (*Amaranthus viridis* L.) plants in the mulberry garden as a trap crop for root-knot nematode was well elucidated. It reduced the root-knot severity because of more susceptibility of amaranthus to root-knot nematode than mulberry (Datta and Datta, 2007). Apart from trap crops, planting cover crops (non-host to the same nematode) also helps in mitigating the incidence of nematodes (Gill and Mcsorley, 2011). Cover crops like cowpea, sunhemp can be cultivated in the mulberry garden to reduce root-knot nematodes, due to the presence of cover crops, nematodes cannot move to another field (Gill and Mcsorley, 2011). Beside mitigating nematodes, cover crops were also known to reduce soil erosion, enhance soil fertility, suppress weeds and many soil-borne pathogenic microbes (Balkcom *et al.*, 2007). Antagonistic plants possess the characteristics of secreting antagonistic root exudates against root-knot nematode was known to reduce the nematode egg masses and their population. Marigold, *Crotalaria*, rapeseed and oats are some of the plant species with well-reported antagonistic activity against root-knot nematode (Govindaiah *et al*., 1991 and Kafle, 2013).

Another way to reduce root-knot nematode is through soil solarization (Tisserat, 2006). It will be done during the summer season by covering the soil with a plastic-film to increase the soil temperature which led to the destruction of nematode egg masses and population upon exposure to excessive temperature (Noling, 2009). The different cultural methods of management of root knot nematode in mulberry with their mechanism of action was mentioned in Table 1.

3.3 Biological Approaches

Managing the pathogen inoculum below the economic threshold level (ETL) can be achieved through various biological approaches like botanicals, plant extracts and biocontrol agents. These are known to combat the pathogen inoculum and enhance the growth and development of host plants (Adesemoye *et al*., 2009 and Sharma *et al.*, 2012).

3.3a Plant Extracts

Plants are the hub of various well-known antimicrobial compounds. These naturally occurring antimicrobial compounds may serve as potential inhibitors of plant-parasitic nematodes and also as an alternative to synthetic nematicides (Nelaballe and Mukkara, 2013 and Muniasamy *et al.*, 2010). Different plant parts *viz.,* leaf, seed, seed kernel, etc., from diverse plants showed anti-pathogenic properties against many plant pathogens. There are well-known reports to claim that, these plant extracts are found to contain varied secondary metabolites *viz.,* alkaloids, saponins, flavonoids, tannins, terpenes, acetylene and glucosinolates (Chitwood, 2002). Extracts of foliar plant parts from diverse plant species were known to possess anti-nematode properties by inhibiting the egg hatching and J_2 juvenile mortality of *M. incognita* (Goswani and Vijayalakshmi, 1986; Adegbite, 2003; Datta, 2006; Pavaraj *et al*., 2010 and Moosavi, 2012).

Foliar application of *Artemisia nilagirica* (Clarke) plant extract products *viz.,* mother tincture (MT) and Cina 200C to mulberry reduced the nematode infection in terms of number of galls and nematode population in roots. Apart from reducing the root-knot infestation it also enhanced the growth (both shoot and root biomass) and development of mulberry plants (Datta, 2006). Leaf and stem extracts of *Ageratum conyzoides* showed greater egg inhibition and J_2 juvenile mortality of *M. incognita*. The anti-nematode properties of *A. conyzoides* extract are attributed to the presence of secondary metabolites like alkaloid, phenol, saponins, tannins, gylcosides, etc. (Asif *et al.*, 2017). Leaf extracts of *Couroupita guianensis, Nepeta cataria, Pentanema indicum, Clitoria ternatea, Passiflora foetida, Datura stramonium, Azadiracta indica, Calotropis procera, Crotalari*a *juncea, Acyranthes aspera* and *Solanum xanthocarpum*

TABLE 1

Cultural approaches for the management of root knot nematode in mulberry

Cultural methods	Mechanism of action	Reference
a) Summer ploughing b) Soil solarization	Exposure of egg masses and juveniles of root-knot nematode to high temperature causes desiccation and death	Sharma, 1999; Tisserat, 2006; Noling, 2009
c) Mulching (Neem and pongamia leaves)	Nematicidal metabolites (Ex. Azadirachtin in neem) affect the egg masses and juveniles of root knot nematode	Javeda *et al*., (2007); Arote and Yeole, (2010)
d) Trap crops	Serves as susceptible hosts and attracts root knot nematodes	Datta and Datta, 2007; Gill and Mcsorley, 2011

inhibited the egg hatching and increased the J_2 juvenile mortality of *M. incognita* in mulberry (Pavaraj 2012; Kumari & Devi, 2013; Nelaballe & Mukkara, 2013 and Asif *et al.*, 2017). Soil application of *Acacia auriculiformis* plant extract and a neem-based product called nemahari effectively inhibited the root galls and multiplication of *M. incognita* in the mulberry garden (Ramprakash and Kshirsagar, 2017).

Apart from leaf extracts, seed and stem extracts of some plants were also reported to possess anti-nematode properties. Soil application of neem (Gupta *et al.*, 2020 and Govindaiah *et al.*, 2003) and pongamia seed kernel extracts (powder form) in pot culture experiments showed greater inhibition of egg hatching, increased larval mortality and reduced the root-knots in mulberry caused by *M. incognita* (Govindaiah *et al.*, 2003). Similarly, dharek seed kernel extract (Gupta *et al.*, 2020), *Ageratum conyzoides* stem extract (Asif *et al.*, 2017) and extracts of *Allium sativum* and *Lasownia inermis* (Naik *et al.*, 2007) were also known to reduce egg hatching and increased the juvenile mortality of *M. incognita* in *in-vitro* studies. Some bioproducts like agniastra (2%) (composed of desi cow urine, neem leaves, tobacco powder, green chilli paste and garlic paste) and also cow urine (10%) were reported as inhibiting egg hatching and effective in increasing the juvenile mortality of *M. incognita* (Gupta *et al.*, 2020).

3.3b Beneficial Microbes against Root-knot Nematode

Secondary metabolites of various microorganisms from diverse ecological niches were reported to have potential antimicrobial activity against a wide range of plant pathogens. Among them, many micro organisms of fungal and bacterial origin were known to possess anti-nematode properties in both *in-vitro* and *in-vivo* studies discussed below.

In-vitro Studies

Secondary metabolites extracted from the beneficial microorganisms in the form of crude extracts or culture filtrates in broth culture contain a diverse group of secondary metabolites like phenols, alkaloids, terpenoids, polyketides etc., which are antimicrobial. Culture filtrates of *Trichoderma viride, T. hamatum* (Narasimhamurthy *et al.*, 2012 and Khan *et al.*, 2020) *Trichoderma harzianum, Aspergillus niger, Pochonia chlamydosporia*, *Fusarium oxysporum* and *Pseudomonas fluorescens* screened against *M. incognita* showed the suppression of egg hatching and also increased the mortality of J_2 juveniles in *in-vitro* conditions (Narasimhamurthy *et al.*, 2012). Similarly, the culture suspension of various biocontrol agents *viz., Trichoderma viride*, *Paecilomyces lilacinus*, *Pseudomonas fluorescens* and *Pasteuria penetrans* were screened against *M. incognita* in *in-vitro* condition. All the four bio-agents effectively suppressed the egg hatching and also the viability of J_2 juveniles of *M. incognita*. Among them, *P. fluorescens* and *P. lilacinus* were superior over *T. viride* and *P. penetrans* in inhibition of egg hatching and *P. lilacinus, P. penetrans* and *P. fluorescens* were superior over *T. viride* in increasing the larval mortality (Popal, 2020).

In-vivo Studies

Bio-control agents with potential anti-nematode activity in *in-vitro* conditions were screened in *in-vivo* conditions to elucidate their efficacy under field conditions. In pot culture experiment, soil application of *T. harzianum* THN1 strain effectively inhibited the egg hatching followed by a reduction in the root-knots and also enhanced the leaf yield (Sukumar, 2004). Similarly, soil application of *Trichoderma viride* (2×10 cfu/g) and *Psuedomonas fluorescens* (1×10 cfu/g) effectively suppressed the number of galls, egg masses and also the population of *M. incognita* in mulberry. Along with that it also enhanced the growth and yield of mulberry by increasing the shoot length, number of leaves and leaf yield (Narasimhamurthy *et al.*, 2011).

Combining the beneficial microbe along with organic manures is reported to reduce the root-knot nematode infestation and also enhanced the growth and development of plants. Soil application of seri-compost (silkworm litter and rearing waste) enriched with beneficial microbes *viz.,* phosphate solubilizing bacteria (*Bacillus megaterium*), *Azotobacter chroococcum* and antagonistic microbes *viz.,*

TABLE 2

Biological approaches of root-knot nematode management in mulberry

Biological methods	Mechanism of action	Reference
Botanicals		
Leaf extracts of *Artemisia nilagirica, Datura stramonium, Azadiracta indica, Pongamia pinnata, Calotropis procera, Crotalaria juncea* and *Acyranthes aspera.*	Plant extracts contains secondary metabolites (alkaloids, saponins, flavonoids, tannins, terpenes, acetylene and glucosinolates) with nematicidal properties.	Chitwood, 2002; Pavaraj *et al.*, 2012; Kumari and Devi, 2013; Nelaballe and Mukkara, 2013 and Asif *et al.*, 2017.
Bio-control Agents		
a) *Trichoderma* spp.	a) Affects nematode egg hatching by secretion of chitinase	a) Sahebani, N. and Hadavi, N., 2008
b) *Paecilomyces lilacinus*	b) Parasitizes the eggs of root-knot nematode	b) Goswami and Mittal, 2004
c) *Pseudomonas fluorescens*	c) Inhibits the egg hatching	c) Narasimhamurthy *et al.*, 2011
d) *Pasteuria penetrans*	d) Hyperparasitism on nematode juveniles	d) Sayre and Starr, 1985

T. harzianum and *Trichoderma pseudokoningii* effectively reduced the root-knot disease in mulberry and also improved soil health (Sharma, 2012). Various plant extracts and biocontrol agents with their mechanism of action against root-knot nematode infecting mulberry were mentioned in Table 2.

Root-knot in mulberry caused by *M. incognita* is one of the major constraints to reducing the leaf yield in both quality and quantity. Even though various management strategies were followed to mitigate the root-knot nematode infestation in mulberry eco-friendly approaches are of prime importance because of the deleterious effect of nematicides on the environment and also toxic effect on silkworms. Various eco-friendly management approaches were reported to have great impetus on the inhibition of root-knot nematode in field conditions. Being a perennial crop it takes several years for developing a resistant/tolerant variety against root-knot nematode in mulberry. Different genotypes and germplasms are having tolerant and resistant reactions against root-knot nematode that can be utilized for root-knot nematode resistance breeding program in mulberry. Cultural practices like deep ploughing during summer condition increases the soil temperature thereby decreasing the root-knot nematode inoculum in the soil. The application of organic manures, neem and pongamia cakes directly affected the root-knot nematode population and also altered the beneficial rhizosphere microbes population. Many botanicals *i.e.*, plant extracts from diversified plant species inhibited the egg hatching and also juveniles of root-knot nematode in both *in-vitro* and *in-vivo* conditions. Exploiting these botanicals commercially for field application will be led to the development of a better alternative to synthetic nematicides. Apart from botanicals, many biocontrol agents efficiently reduced the root-knot nematode infestation and are also enhanced the growth, development and yield parameters in mulberry. All these eco-friendly approaches can be followed to mitigate the root-knot disease incidence in mulberry and further research work is required to exploit the many biocontrol agents against root-knot nematode in field conditions.

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