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Review Article

Root-knot nematode (*Meloidogyne incognita*) and its management: a review

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ABSTRACT

Root-knot nematode (RKN) *Meloidogyne incognita* stands out among the most harmful polyphagous endoparasite causing serious harm to plants, and distributed all over the globe. RKN causes reduced growth, quality and yield along with reduced resistance of the host against biotic and abiotic stresses. Infective second stage juvenile enters host roots with the help of the stylet and becomes sedentary getting into the vascular cylinder. Dramatic changes occur in host cells, making a specialized feeding site, induced by the secretion of effector protein by RKN. *M. incognita* can be controlled by nematicides, biocontrol agents, botanicals essential oils and growing resistant cultivars. Nematicides are no longer allowed to use in many parts of the world because of environmental hazards and toxicity to humans and other organisms. Researchers are concentrating on searching suitable alternatives to nematicides for effective management of *M. incognita*. This review mainly tries to explain the biology of M. incognita and different management options recommended in recent years. However, an effective and economical management of *M. incognita* remains an immense challenge.

Keywords: Life cycle, management, nematicides Root-knot nematode (RKN).

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INTRODUCTION

Nematodes are classified under large phylum Nematoda, which covers un-segmented roundworms. Nematodes are cosmopolitan in nature, found in almost all habitats around the globe. They can adapt to diverse and extreme conditions of cold to hot areas of deserts. According to the feeding habits and lifestyles, plant parasitic nematodes are grouped into ectoparasites and endoparasites. Those nematodes that feed by inserting the stylet into root cells being outside on the root surface are ectoparasites, but those penetrating host cells and feed from inside are endoparasites (as reviewed by Escobar *et al.*, 2015). Root-knot nematodes (RKN) fall under group sedentary (sessile) endoparasite. They got their names from the distinct structure they form in the roots of the infected plants: the galls or knots. Root-knot nematodes fall under genus *Meloidogyne* which is derived from the Greek word

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meaning apple-shaped female. *Meloidogyne incognita, M. hapla, M. javanica*and *M. arenaria*are the four commonly found species which comprise up to 95 percentage of all RKN (Dong *et al.*, 2012).

Matured female lay egg masses on the surface of the root. Sometimes eggs are embedded in gall or plant tissue, which are usually up to 1000 in numbers. First stage juvenile (J1), after the embryogenesis molts within the egg to the infective juvenile of the second stage (J2), that is hatched from the egg. Hatching of *Meloidogyne incognita* mainly depends on suitable moisture and temperature conditions because of their broad host nature and no stimulus from the host plant is needed. In some conditions, root diffusates and hatching response can be influenced by generation number (Curtis *et al.*, 2009). Second stage juvenile (J2) penetrates the root of host plant piercing plant cell wall with the help of the stylet. RKN moves between cortical tissue and cells and gets into the plant vascular cylinder and becomes sedentary (Hussey & Grundler, 1998, Abad *et al.*, 2008). The stylet is also used for secreting secretions from the esophagus and taking nutrients. J2 provokes the dedifferentiation of surrounding 5-7 cells into multinucleated and enlarged giant cells (figure 1).

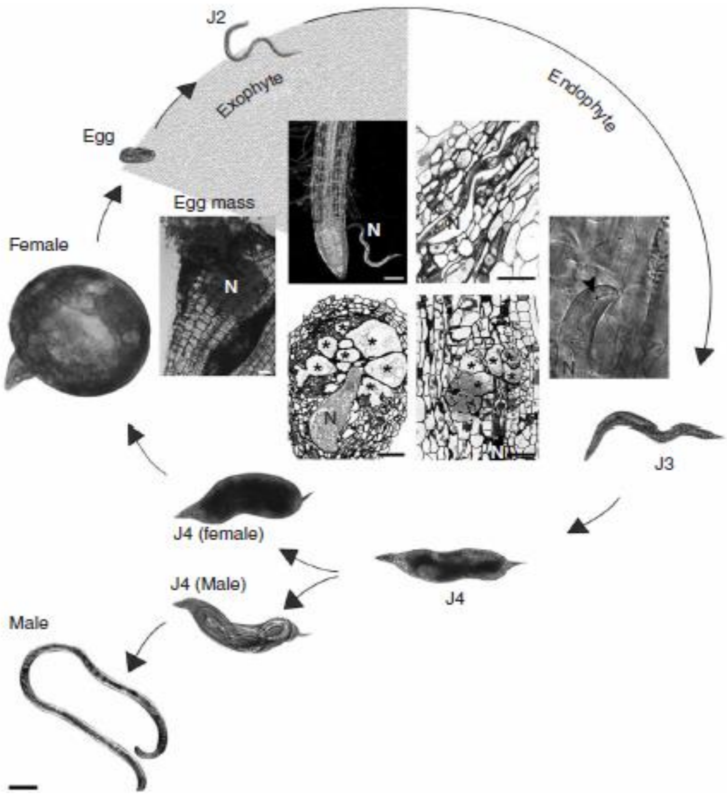

Figure 1: Parasitic life cycle of *Meloidogyne incognita* (Source: Abad *et al.*, 2008)

These cells act as a nutrient source to the nematode (figure 1). RKN passes through three molts, Juvenile third stage (J3), fourth stage (J4) and adult. Sometimes, males are developed and come out of the host roots. They are believed to have no role in the reproduction process. Females are pear shaped and release eggs on the surface of the root (Abad *et al.*, 2008). Roots damaged by the RKN cannot make use of water and nutrients efficiently. Infection in mature

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plant causes a decrease in yield, but infection in the young ones may be lethal (Anamika & Sobita, 2012).

Root-knot nematodes are considered as one of the economically important pests that cause damage to plant growth and reduce yield. A loss of 100 billion $every year is estimated to be caused by *Meloidogyne incognita* alone worldwide (Mukhtar *et al.*, 2014). RKN damage results in poor growth, reduce quality and yield and also decrease the resistance of crop against drought and diseases. Total crop failure can occur due to high damage of RKN (Anamika & Sobita, 2012).

Diseases caused by RKN are not epidemic, but there is a slow decrease in production, year after year. Symptoms may be scattered, i.e. some area may be affected and no signs in other areas. We may get confused about RKN damage looking at the above ground parts with a mineral deficiency or stress. Due to the sedentary endoparasitic nature, total control of RKN seems very difficult. A large number of methods for the management of RKN such as resistant cultivars, soil solarization, use of chemicals and biological control have been tried across the globe. The uses of chemicals are effective, expensive and lead to environmental side effects (as reviewed by Terefe, *et al.*, 2008). In the present context, the emphasis is put on biological means of controlling RKN. Bio-control agents can offer economic and eco-friendly options for the management of RKN. Many researchers have proposed eco-friendly means of controlling RKN such as the use of resistant germplasm (Gisbert *et al.*, 2012).

This review tries to show the recent advances in understanding the life cycle of RKN and what search for different management practices that are found effective against it. The objective of this study is to understand in brief about the biology of RKN and more comprehensively on the practices that are effective for the management of RKN.

Life cycle of Root-knot Nematode (RNK)

RKN reproduces mainly by mitotic parthenogenesis. Matured female lays eggs in gelatinous masses that are composed of a glycoprotein matrix. Gelatinous masses, produced by rectal glands, are secreted before and at the time of laying eggs. The matrix protects the egg from extreme environmental condition and predation. The eggs mass is found surrounded within the gall tissue or on the surface of root galls. Initially soft and clear eggs mass gradually changes to firm and dark brown (Moens *et al.*, 2009). RKN has four juvenile stages and an adult stage with four molts in-between the stages. They replace their cuticle during molting. First stage juvenile (J1), molts into second juvenile stage (J2), remains inside the egg. J2 cannot live long in the absence of a host. J2 hatches out when the environment is favorable. J2's, are attracted by plant roots (Curtis *et al.*, 2009), to reach the vascular cylinder of the host plant by piercing through growing tip and moving through intercellular spaces (Caillaud *et al.*, 2008a). There RKN becomes sedentary and draws water and nutrients from a few root cells that are transformed into a special feeding structure called giant cells (GC). After three molts J2 mature into adults. Sex determination is environmentally dependent. In adverse environmental conditions and during the scarcity of nutrition, more males are produced. Males are motile during the third molt and leave the host. Female remain sedentary and produce eggs on the root surface in a gelatinous matrix (Papadopoulou and Triantaphyllou, 1982).

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Table 1. Life stages duration in RKN (*M. incognita*) on noni (*Morindacitrifolia*).

Life stage	Duration (Days)
Second stage juvenile (J2)	1-5
Third stage juvenile (J3)	6-8
Fourth stage juvenile (J4)	9-12
Adult male	23
Adult female	27
Total life cycle	25-30

(Source: Kavitha *et al.*, 2011)

RKN-Host interactions

Plant cell walls, the outer protective layer, provide mechanical support to the plant cell against the intracellular pressure. Flexible cell wall structures are needed to respond to various development conditions, abiotic and biotic stimulus (Wieczorek, 2015). Plant cell wall structure and its chemical composition act as a barrier to many pests including nematodes. RKN has a stylet which is used as a tool for forcing itself through the plant cell wall. In spite of this, host cell walls are digested by the protein secreted by RKN. This facilitates successful penetration into the root and migration inside the plant cell and become sedentary. Effector proteins are secreted into the plant cells from the dorsal gland of RKN to start the development of feeding site in the plant root. RKN takes nutrients from this special feeding site. The effectors force the host to benefit RKN and avoid plant defense (Quentin *et al.*, 2013).

Nematode sets up a close relationship with the host during infection. GC constitutes many nuclei, which results from repeated nuclear division without cell division. Initially, a second nucleus is seen as a sign of interruption of cell development by RKN. The condition seems that cell will divide into two, but results in a cell with two nuclei due to the disturbance in the formation of the cell plate (Caillaud *et al.*, 2008b). Division of nuclei goes on until GC contains around 100 nuclei. The size of GC may be up to 400 times bigger than regular root vascular cells (Abad *et al.*, 2009). RKN constantly extracts cytoplasm from the infected cell, converting it as a metabolic sink for the host. Division of cell nearby RKN and GC give rise to a proliferation resulting into the formation of knots in the root of the host. Defense response is activated in the host during damage by the RKN. According to Kyndt *et al.* (2012), wound responsive jasmonate and ethylene pathways are induced upon infection of nematode in rice. A strong decrease of hormone pathway related to defense mainly ethylene and salicylate has been marked in GC, developing galls and surrounding tissues.

Effective management techniques of RKN

Effective management of RKN depends mainly upon the initial population of nematodes and host plant. Complete control or eradication of RKN is not possible. Many management techniques have been experimented and recommended such as cultural methods, physical, chemical, biological, host plant resistance and integrated management approaches. During this review, a literature study is conducted to investigate the different methods. Their practicality and relative effectiveness will be discussed.

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1. **Synthetic nematicides and botanicals**

Chemical control makes use of different formulations of inorganic chemicals that kill or obstruct the reproduction of RKN. Nematicides having active ingredients as methyl bromide, whichhas been banned for use in many parts of the world, because of its effects on other organisms (UNEP, 2006). As mentioned by Radwan *et al.* (2012). Cadusafos, an organophosphate based nematicide, is one of the most widely used non-fumigant to control nematodes. Nematicidal treatments inhibit egg hatching and development of nematodes because of both nematicidal effects on nematodes and by inhibiting their penetration in the host. Cadusafos 1% was found most effective on egg inhibition and caused 100% mortality of juveniles. Cadusafos significantly minimized RKN population at all stages of development when applied as a curative or protective measure (Safdar *et al.*, 2012).

Emamectin benzoate (avermectin obtained from bacteria *Streptomyces avermitilis*) and Cadusafosis used in greenhouse and field trials of tomato for their efficacy against *M. incognitan* (Cheng *et al.*, 2015). Emamectin benzoate is also used as a biological pesticide. It has a broad spectrum of insecticidal activity. Emamectin benzoate as soil application at the rate of 150 gm/ha was the most effective in lowering the gall formation by *M. incognita* while maintaining good plant growth. It was found more effective than standard nematicideCadusafos. This suggests Emamectin benzoate is a good nematicide having high nematicidal activity on *M. incognita*.

The soil fumigants 1, 3-dichloropropene (1, 3-D) and Chloropicrin (CP) can be used as an alternative to methyl bromide. 1,3-D (92% EC) and CP (99.5% liquid) at the rate of 150 and 250 kg/ha was found effective in controlling nematode population and lowering root galling indices (Qiao *et al.*, 2015).

Melia azedarach commonly called chinaberry exhibit a broad range of biological activities of pharmaceutical and agricultural use (Ntalli *et al.*, 2011). Matured fruits of *M. azedarach* were grinded and aqueous extracts are prepared. Application of crushed fruits of *M. azedarach* at the rate of 30 and 60 g/kg showed properties like that of insecticide fenamiphos (0.02g a.i./kg) in terms of reducing reproduction rate as well as the population of nematodes in root and soil. Aqueous extract of *M. azedarach* is rich in carboxylic acid, alcohols and aldehydes showed nematicidal activity against *M. incognita*; *M. azedarach* control RKN in cucumber directly, but also reduced the activity of antioxidant enzymes and activate the host defense in an indirect way (Cavoski *et al.*, 2012).

2. **Nematophagous bacteria**

Pseudomonas fluorescens, is a common rod-shaped, gram-negative bacterium produces an antibiotic 2,4-diacetylphloroglucinol (DAPG) and suppresses plant-parasitic nematodes. Seeds treated with Wood1R, a D-genotype strain of DAPG producing *P. fluorescens* lowered the population of *M. incognita* in corn, cotton and soybean in steam-heated soil. In natural soil Wood1R suppressed a moderate level of nematode population: 38% to 50% in comparison to control. The reason for such a low level of suppression of *M. incognita* in natural soil might be due to other microbes suppressing root colonization and/ or production of DAPG by the bacteria (Timper *et al.*, 2009). Hasan and Abo-Elyousr, (2011) also reported that *P. fluorescens* causes about 45% mortality of J2 of RKN in tomato. *P. fluorescens*incombination with *P. lilacinus* and *P. guilliermondi* have a lethal action on nematode and also improve plant growth and stimulate systemic resistance in host. *P.*

fluorescens alone or mixed with *Trichoderma viridae* at the rate of 10g/plant each reduced the effect of *M. incognita* when compared with carbofuran in mulberry (Muthulakshmi *et al.*, 2010).

BioNem, is a chemical WP (wettable powder) formulation of a bacterial species *Bacillus firmus* which, was evaluated in the laboratory, greenhouse and field conditions against *M. incognita* on tomato plants by Terefe *et al.* (2008). In laboratory, 24 days after treatment, hatching was reduced from 98% to 100% with 0.5%, 1%, 1.5% and 2% aqueous suspension of BioNem. Treatment of J2 with 2.5% and 3% concentration of BioNem completely inhibited the mobility 24 hours after treatment. Gall formation was reduced by 91% in the greenhouse treated with BioNem at the rate of 8 g/pot containing a tomato seedling with 1200 cc soil. Nematode population was reduced by 76% and egg by 45%. BioNem WP controls nematodes effectively and a single application is enough for a season.

3. Nematophagous fungi

Trichoderma longibrachiatum, nematophagous fungi, is one of the important biological control measures for *M. incognita*. An experiment conducted by Zhang *et al.*, (2015) showed that conidia suspension of *T. longibrachiatum* had a parasitic and lethal effect on J2 of *M. incognita*. More than 88% of J2 after two weeks of treatment were inhibited and paralyzed with the concentration of 1.5×10^7 conidia/ml. Conodia of *T. longibrachiatum* attached on the surface of J2 germinates with many hyphae and penetrates the integument. J2 gets deformed and starts to dissolve by the metabolite of fungus. Affokpon *et al.*, (2011) also reported *T. asperellum* T-16 suppressed J2 of RKN by 80% and *T. brevicompact*um T-3 suppressed egg production by 86%.

*Syncephalastrumracemosun*and *Paecilomyceslilacinus* were evaluated in vitro for their ovicidal and larvicidal properties and their combined effects were tested to control *M. incognita* in cucumber. A combination of *S. racemosun*and *P. lilacinus* at 50% concentration reduced egg hatching by 70% and fewer gall and nematodes were observed under pot condition. Soil drenching with the combination of *S. racemosun*and *P. lilacinus*controled*M. incognita* significantly, because of their direct parasitism on nematodes egg (Huang *et al.*, 2015).

4. Essential oils

Essential oils are secondary metabolites with distinctive odor and flavor found naturally in a large number of plants. Essential oils from plant sources are believed to have nematicidal properties (Barbosa *et al.*, 2010). The use of essential oils can be considered as alternatives to synthetic fumigants, because of high volatility, and are environmentally friendly. Recently essential oils of *Mentha*, *Cymbopogon* and *Eucalyptus* were proved to have toxic effects to J2 of *M. incognita* (Ntalli *et al.*, 2010). Laquale *et al.*, (2015) tested the effectiveness of soil fumigation with the essential oils derived from *E. citriodora*, *E. globules*, *M. piperita*, *Pelargonium asperum* and *Rutagraveolens*on potted tomato in the greenhouse. Essential oils were applied at the rate of 50, 100 and 200 μL/kg soil. Oils from *E. globules* and *P. asperum* reduced multiplication of nematodes and gall formation on roots at all applied rates while *R. graveolens*, *M. piperita* and *E. citriodera* showed effects at levels of 100 and 200 μL/kg soil.

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5. Resistance cultivar

Use of resistant cultivars is one of the important alternatives for management of RKN. This management option is environmentally friendly and easy to use by the growers. Cultivars resistant to RKN will definitely have a better crop yield in comparison to cultivars that are susceptible. Resistant cultivars can be used in combination with other management options as the use of botanicals, biological control, cultural control etc. in the form of integrated nematode management. Mukhtar *et al.* (2014) identified some cultivars of okra (*Abelmoschus esculentus*) like Arka, Anamika, Ikra-1 and Ikra-2 that showed moderate resistance to RKN as these were less damaged by RKN in comparison to other susceptible ones. Gisbert *et al.* (2013) tested for resistance genotypes in pepper and also the associated N and Me genes. These genes may not be much active during high temperatures. The genotypes having resistant genes against RKN can be used in breeding programs to develop new resistant cultivars. Planting of these resistant cultivars can be important in controlling RKN and their infections.

Discussion

Different papers were selected based on different aspects of RKN. Reviewing life cycle of RKN, its interaction with host plants gives a clear idea about the growth and development of infection on host. RKN is a greater problem for agriculture in many parts of the world. The ultimate goal for management of RKN is to save the plant from its infection, prevent secondary infections and attain highest crop yield with minimum cost. There is an immediate need to develop suitable management strategies for RKN. Some preventive measures are not fully effective, but are useful as soil solarization, use of healthy seeds, crop rotation, clean cultivation etc. These may not be applicable because of many constraints as temperature requirement, time, labour intensive, and expensive.

Generally, the uses of botanicals are considered more sustainable due to less environmental effects and less effects on other organisms when comparing synthetic nematicides. In the recent world, ecologists and scientists are recommending the use of botanicals because of being more environmentally friendly with the minimum risk associated with it. Essential oils extracted from many plants have nematicidal properties and can be used as a replacement for synthetic fumigants can also be used. One of the most efficient chemical for controlling RKN, methyl bromide,which was banned for use by the EU in 2005. Nematicides highly reduce the population of RKN in the soil in earlier juvenile stages, but in later stages when symptoms are developed, they are unable to stop yield losses. Excessive use of chemicals also has a risk of development the resistance of the pathogens.

Biological control involves the use of living organism alone or in combination with other organism. Organisms that are antagonistic to RKN have been used by many researchers. Most of the biocontrol agents efficiently control RKN at a specific stage of their life cycle. Most commonly nematophagous fungi and bacteria are used to control RKN. Some commercial products made from biocontrol agents are used for the management of RKN. Infection can be lowered by affecting the juveniles of RKN. Decreasing the population of RKN is difficult, mainly of those having multiple generations in a growing season. Only multiplication of the RKN is lowered, but control of eggs and females doesn't necessarily prevent root infection and damage to the host. The female of RKN remains inside the host and is sedentary, so all the rhizospheric fungi cannot parasitize it. Fungi may not be able to

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ruin the egg of RKN when the temperature is high, because high temperature favors early hatching of eggs. Biological control till now not a cornerstone in controlling RKN because of the quantity of material required to gain a significant effect for application in large areas and production of inoculums of nematophagous bacteria and fungus. However, on long time duration added value of the biological control is high because of hygienic environment, no contamination of food and water, good health of humans and other organisms.

Conclusion

RKN are considered one of the serious pests that cause huge economical loss to different agricultural plants and reduce the production. Losses range from few percentages to total failure of crop. RKN are very difficult to control firstly as being a soil borne pathogen and secondly having a wider host range. Many chemicals are tested and used widely to control the RKN but they are proven to be toxic, expensive and cause serious effect to nature. The other alternative as biological control, botanicals and resistant cultivars can be used for the management of RKN and reduction of the damage caused. The attraction of farmers towards the biological control measures, researches on understanding the molecular basis of fungal and bacterial pathogenic mechanism on RKN must be continued to identify economical and low volume requiring bio-control agents to control RKN.

Authors' contributions

SS drafted the concept. BT and JS revised and finalized the paper.

Conflict of interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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