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Chapter

# Root-Knot Nematodes a Major Peril to Protected Cultivation System in India: Current Status and its Management

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## Abstract

Growing of vegetable crops under protected conditions are relatively, an innovative technology and most popular among farmers throughout the country. In last few decades protected cultivation has shown potential enhancement in horticultural production. The southern root-knot nematode, *Meloidogyne incognita*, is an emerging nematode under protected conditions. This nematode can cause chlorosis, stunting and reduce yields associated with the induction of many root galls on host plants. Root-knot nematode severely affect the plant root system by inducing specialized feeding cells i.e., giant cells in the vascular tissues. Recently, this nematode has been considered as a worldwide menace for combat root-knot nematodes, integrated nematode management strategies such as soil solarization, biological control, organic amendment, crop rotation, field sanitation, and fumigants have been developed and successfully used in the past. Here, in this book chapter discussed on biology and life cycle, control measures and proposed future strategies to improve *Megalaima incognita* management under protected conditions.

**Keywords:** protected cultivation, root knot nematode, vegetable crop and integrated nematode management

## 1. Introduction

Cultivation of crops in protected structures is relatively a new or advance technology, growing crops in controlled environments (temperature, humidity, light and such other factors can be regulated as per requirement of the crop). It is popular among farmers/growers globally. Commonly used structures are forced ventilated greenhouse, naturally ventilated polyhouse, high-tech polyhouse, insect proof net house, shade net house, plastic tunnel and mulching. Protected structures may be demarcated as "Alteration of environmental condition in such a way to accomplish maximum growth and yield" [1]. Recently, incipient technology for raising high value crop in the country and it has very decent potential in semi-urban areas (nearby cities). Altered environmental conditions, bounces manifolds increase in yield per unit area. Modernized protected cultivation are very popular among growers in all over world and approximately 405000 ha area covered under protected cultivation globally [2] as compared to India 30000 ha area under protected cultivation, is still in infancy stage [3]. In India, protected structures are being initiated by National Horticulture Mission to increase per capita income of framers. Protection of pest and diseases under controlled environmental conditions, farmers are getting very good returns from this technology. Globally, among polyhouse cultivated crops, *Cucumis sativus* L. is an important vegetable and second most popular crop.

In polyhouses, three types of crops are grown, *viz*. vegetable crops such as cucumber, capsicum, tomato, ornamental crops such as, carnation, roses, gerbera, chrysanthemum and fruit crop like strawberry. Growing vegetables and flower crops under protected cultivation is receiving utmost attention and gaining popularity among farming community across the country. The ideal conditions provided by protected cultivation and continuous availability of the host plant round the year often results in high population buildup of soil borne pathogens including plant parasitic nematodes. However, Plant parasitic nematodes are becoming a major constraint in production of the horticultural crops under protected cultivation in India. Root-knot nematode, *Meloidogyne* spp. has to be the major plant parasitic nematode under protected conditions [4]. There are various management strategies viz., soil solarization, biological control, organic amendment, chemical and integrated nematode management practices have been followed for the management of the plant parasitic nematodes.

Protected structures aided crops with altered climatic conditions to get supreme yield potential than open field by shielded from adversities [5]. Ancient records, during 14–37 AD, when Roman Empire was controlled agricultural production, certain limited structures were present. Nevertheless, commercial protected cultivation had been initiated in England trailed by France, Netherlands, Japan and China at ending of eighteenth and nineteenth century [6]. Charles Lucien Bonaparte, French botanist (1803–1857) are accredited for making the first modern greenhouse (http://english.reachgreenhouse. com/news_view_32_105.html). High value agricultural crops are mostly preferred for protected structures to optimize production cost as well as reduced biotic and abiotic stresses.

## 2. Prevalence of root-knot nematode under protected cultivation

Root-knot nematode, *Meloidogyne* spp. are foremost important parasite in protected cultivation and having ability to parasitize on most of the crops. Around 232 plant species attacked by root-knot nematodes including vegetables, fruits, fiber, ornamentals, medicinal, cereals and weeds also. Root-knot nematodes are obligate parasites causes severe damage to vegetable crops (**Figures 1**–**3**), leading to major yield reductions and significant economic losses worldwide [4, 7–9]. Root-knot nematode development and fecundity are very high and very with populations/races. Second stage juveniles randomly move in soil and attracted by chemicals released from host roots thus seek to infect roots. Meristematic zone is the most preferred site for penetration of second stage juveniles. Galls on roots are the diagnostic symptom, results from hypertrophy and hyperplasia after nematode feeding. As endoparasites, all stages were found in root tissues, except vermiform male and second stage. Optimum temperature required for development was 15 to 30°C. One generation, from egg to egg, was completed within 25 to 30 days. In a conducive environmental condition, nematode population build-up increases rapidly and reaches as high as 20–25 eggs and juveniles per g soil.

Root galls are the most characteristic symptom of root-knot nematode infection (**Figure 1**). As vascular feeder, destroy the xylem and phloem, ultimately translocation of water and nutrient uptake was debilitated. Due to poor transportation, above ground symptoms such as yellowing, wilting, poor fruiting has manifested on plants in patches. These patches gradually increase every year as inoculum has increased.


**Figure 1.**
*Cucumber crop infested wish root-knot nematode, Meloidogyne spp. under polyhouse conditions. A) above ground symptom B) below ground symptoms. (Source: Original photos).*

## 3. Interaction of *Meloidogyne* spp. with other microorganisms

Nematode have long been alleged of playing a bigger role in plant disease scenario with other microorganisms like fungus and bacteria rather than alone. Different role has been played by nematode in complex diseases such as aggravator, predisposer, vector etc. nematodes may act as wounder, host substrate modifier, rhizosphere modifier and vector to make the environment more conducive for development of other secondary pathogens. In complex diseases, root-knot nematode with wilt and root rot fungus causes grater damage to susceptible plants as compared to each pathogen alone [10, 11]. Host physiology has been altered by nematode for secondary pathogens results, complete failure of crop in some instances.

Nematodes provide ready avenues for entry of secondary microbes. Besides avenues, biochemical changes have been initiated in nematode infected plants and enriched giant cells also favor the wilt causing fungi. Seedling mortality was

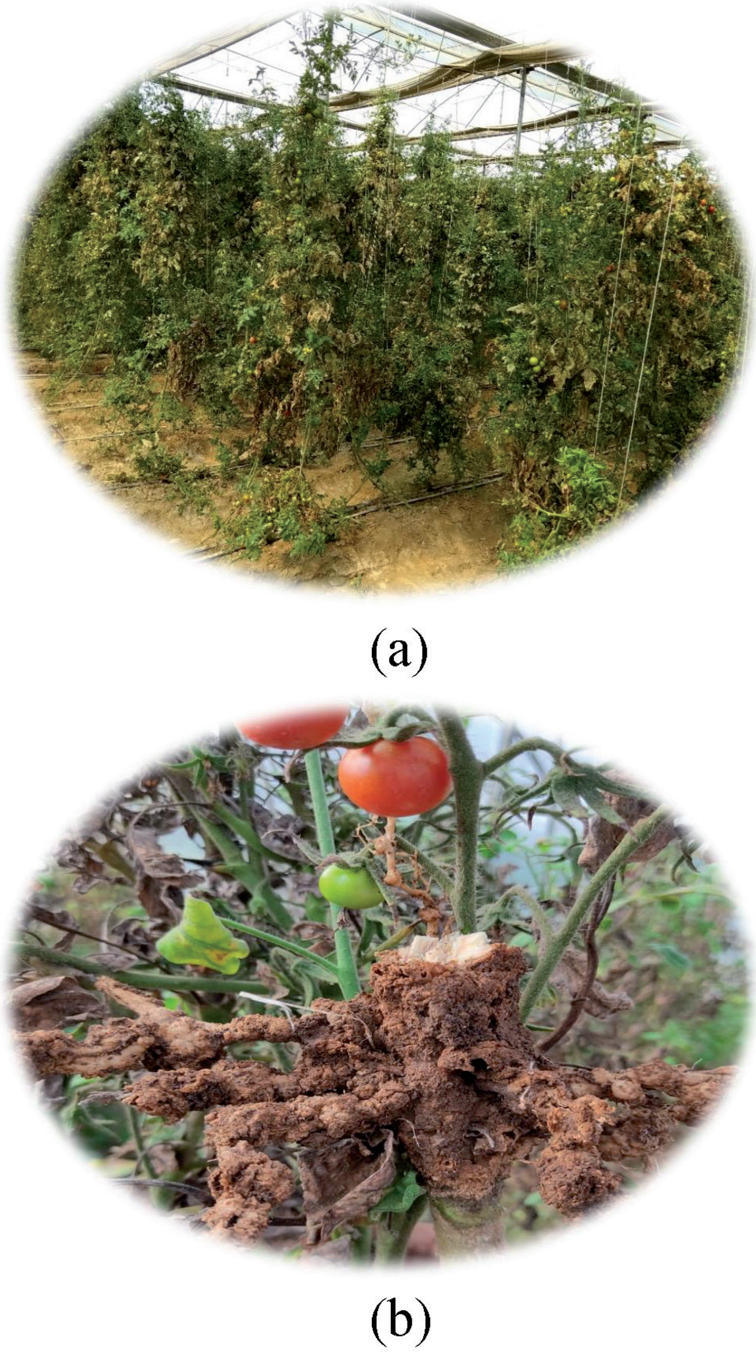


**Figure 2.**
*Tomato crop infested wish root-knot nematode, Meloidogyne spp. under polyhouse conditions. A) above ground symptom B) below ground symptoms. (Source: Original photos).*

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preponed by about a week due to interaction with fungi with nematode. Nematode may also play the role in resistance braker for other pathogens. Some varieties lost the resistance against fungal pathogens in the presence of root-knot nematodes. Due to disease complexes sometimes complete crop failure faces by growers.

## 4. Reasons for multiplication of root-knot nematode under protected cultivation

### 4.1 Moisture

Moisture is the foremost important factor for multiplication of nematodes. Continuous moisture availability around root zones through drip irrigation under

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(a)

(b)

**Figure 3.**
*Capsicum (Bel pepper) crop infested wish root-knot nematode, Meloidogyne spp. under polyhouse conditions A) above ground symptom B) below ground symptoms. (Source: Original photos).*

polyhouses is responsible for fast nematode build-up and movement as compared to open field where flooded and dry conditions prevail. Continuous moisture availability flare-up the nematode population and more infectious.

### 4.2 Temperature

Temperature affects overall life profile activities of nematode such as hatching, movement, reproduction, development, and survival and also the host plant. Optimum temperature range for survival of plant parasitic nematodes is 15-30°C and become inactive or less active from above and below temperature conditions or may lethal for nematode. Under polyhouses optimum range of temperature for nematode build-up exist. Under optimum moisture and temperature conditions in polyhouses nematodes are able to complete several generations in less period of time as compared to open field conditions.

## 5. Continuous cultivation of susceptible host

Crops grown under polyhouses are generally susceptible to nematode pest such as tomato, cucumber, ornamentals etc. due to high economic value monoculture has been adopted by growers. Intensive monoculture of susceptible crops leads to nematode build-up and multiplication rate. All three conditions, susceptible host with favorable microclimate favor fast build-up of nematode population and once it introduced it is very difficult to get rid from this.

## 6. Current management approaches

Favorable climatic conditions concentrated majority of the protected structures in the regions between 25° and 65° latitude [12]. Solar irradiations and temperature is low at higher latitude, that's wants maintenance of humidity and temperature and the conditions created favor the pest incidence. Intense solar irradiation at lower altitude persuades stress in the crops rendering them susceptible to pest incidence [12]. Irrespective of the diverse protected structures and materials for buildup, the microclimate inside the protected structures favor the multiplication of pest and diseases including plant parasitic nematodes [13]. Henceforth, it become extremely difficult to manage the nematode pest from single management options. Keeping in view of this, integration of all available management techniques/tools for better resolution of the nematode pest. The integrated strategies for control of plant parasitic nematodes can be based upon two basic principles: 1) preventive measures and 2) on-farm techniques. Preventive measures avoid the introduction pest species in newer areas and second one is based upon control measure (cultural, biological and chemical) adopted by growers, to reduce pest population below ETL.

## 7. Preventive measures

Preventive measures are adopted to avoid the introduction of nematode pest in newer areas where nematode problem not exist before. Some practices have been adopted as preventive measures to control the spread of nematode. New or emerging species spread has been checked by regulatory methods to avoid the introduction in newer areas. Soil testing are mandatory for all the farmers before erection of polyhouses, green houses and net houses for plant parasitic nematodes. Entry points for protected conditions should also contain sanitizing stations for hands, shoes, boots, tools, and other equipment. Nematode free transplanting material is one of the important methods to avoid the nematode infestation under field conditions. Always use nematode-free transplants or plants that build upon soilless substrates from production are increasingly used to exclude soil borne species of nematodes, but also to promote the plant establishment and crop production.

Raising of crop on soilless media: One of the most important method to prevent spreading of nematodes in nematode free areas through growing of nursery crops in soilless media such as organic growing media: peat, coir, bark, sawdust, compost; inorganic: rockwool, perlite, pumice, sand, vermiculite.

## 8. Curative measures

Curative measures are used to reduce the nematode population below economic threshold level in nematode infested areas so, growers can get maximum returns.

Sanitation can minimize the nematode problems from polyhouses include rapid destruction of infested plant debris and weeds after harvest.

### 8.1 Soil solarization

Soil solarization is a most effective method to reduce the nematode population in hot weather areas (temperature around 40-50° c). In India, northern conditions are best to adopt this practice to reduce nematode infestation under polyhouses. Transparent polyethylene plastic (25 μm thick LLDP) mulch is used to cover the moist soil for 4–8 weeks in the month of May–June [14–17]. Green house effects have been created under transparent polyethylene sheet leading to higher temperature was lethal to nematode.

### 8.2 Crop rotation and inter cropping

Cultural practices are non-chemical method such as crop rotation with resistant cultivars or non-host crops to reduce pest population. Rotating or inter cropping tomato/ cucumber with non-hosts such as garlic (*Allium sativum*), Marigold (*Tagetes* sp.) (**Figures 4** and **5**), lettuce, radish, cabbage and cauliflower could reduce root knot nematode populations in soil. Few options are available in protected conditions to grow non-host crop, so, resistant cultivars are a very good option under protected structures.

### 8.3 Resistant cultivars

Resistant cultivars are one of the convenient options against plant parasitic nematodes. Grafting of commercially desired susceptible cultivars on resistant rootstock is a trending method among vegetable crops under protected conditions [18]. Resistant rootstock of brinjal wild relatives, *Solanum toxicarium*, *Solanum sisymbriifolium* and *S. torvum* have been grafted by commercial tomatoes, noticeable reduction in galling was observed [19]. Various grafted rootstock of melon and capsicum were produced that confirmed extraordinary results in minimize root galling in the greenhouses [20].

**Figure 4.**
*Cucumber intercrop with marigold for the management of root-knot nematode under polyhouse conditions.*

**Figure 5.**
*Marigold crop rotation with cucumber/tomato for management of root-knot under polyhouse conditions.*

### 8.4 Organic amendments

Enormous organic amendments are used for suppression of plant parasitic nematodes in protected cultivation. Suppression efficacy of organic amendments depends on the active ingredient and their concentration. Non-edible oil cakes of Neem (*Azadirachta indica*), castor (*Ricinus communis*) Karanj (*Pongamia glabra*), Mahua (*Madhuca latifolia*) etc. are used for management of rot-knot nematode in protected cultivation [21]. Other organic substances like FYM, vermicompost, slurry, green manure etc. are also effective for suppression of PPNs.

### 8.5 Biological control

Higher efficiency, targeted results, environmentally sound and local acceptability among the growers gain much popularity of the bio-agents in recent era. Egg parasitic fungus- *Paecilomyces lilacinus, Pochonia clamydosporia,* antagonistic fungus- *Trichoderma viride, T. harzianum*, VAM fungus- *Glomus* spp., bacterial parasite- *Pasteuria penetrans* and PGPR bacteria- *Pseudomonas fluorescence* is used as potential bio-agents against plant parasitic nematodes [17, 22–24]. Bio-agents enriched organic amendments are very effective strategy to control nematodes in protected cultivation.

### 8.6 Chemical nematicides

Till now, there are not a single nematicide registered for protected cultivation use in India. Thus, the growers depend on other integrated pest management practices for nematode management under polyhouses. Combination of all preventive, curative measures to control nematode under polyhouses is an effective strategy and locally adopted by growers.

## 9. Novel methods of resistance to root knot nematode under protected conditions

Wide susceptibility range, fast multiplication and cause potential treat at low density are the main constraints for management of root-knot nematode under protected structures. Recently, genetic engineering has made it possible to express and incorporate heterologous and indigenous protein from one to other organisms and develop heightened pest resistance in plants. Genetic engineering approaches has made natural resistance with synthetic resistance may be the auspicious tools for management of nematode in tomato production [25–27].

RNA Interference (RNAi): RNAi has emerging tool to downregulate gene activity and recognized efficient tactic against root-knot nematode [28]. RNAi first performed for *Caenorhabditis elegans* and it was used for gene silencing by overwhelming their expression in a plant parasitic nematode [29]. Nematode feeding site formation gene has been silenced by using dsRNA or siRNA that elicit a systemic RNAi response [30]. Root-knot nematode produces effector proteins determined by parasitism genes, and these effectors epitomize the molecular interface between the nematode and host [28]. Effectors secreted in nematode esophageal glands play perilous roles in parasitism [31].

Exploiting Efficient Genome Editing Using the CRISPR-Cas9 Technique: The advancement of the clustered regularly interspaced short palindromic repeats (CRISPR) technology has become a commanding alternative to gene silencing [32]. Foreign DNA sequences has incorporated host loci to produce short crRNAs (CRISPR RNAs) that direct sequence-specific cleavage of homologous target dsDNA by Cas endonucleases [33]. Recently, documentation of pathogen and host novel genes responsible for infection help in developing the CRISPR technique for improving the resistance to Meloidogyne spp. under protected systems.

Advantages of protected cultivation

- Higher productivity and higher income
- Quality produce
- Off season or round the year cultivation
- Hardening of tissue culture plants
- Better management of insect pest
- Less use of chemicals
- Efficient use of resources

## 10. Downsides of polyhouse cultivation

In spite of protected structures crops grown under these structures are not fully protected from insect pest. Hostile environment, intensive or mono cropping, availability of moisture (drip irrigation) and poor hygienic conditions are increasing the pest problems mainly nematodes under protected environment [34]. Among plant parasitic nematodes root-knot nematodes is the important parasite under polyhouses [4]. Once nematode introduced in the protected cultivation, it's impossible to eradicate the nematode problem. It can build up in less time and causes huge number of losses among the crops. Major source of adulterations in protected structures are planting material, soil and pooting media, water and general cleanliness.

## 11. Conclusions and future directions

Recently, nematode and soil borne pathogens under protected structures paid much attention. Till now efficient management practices under protected structures are very less and are not uses due to certain limitations. Researchers has focused on the environmentally sound conventional and modern management practices under protected structures. Biological and ecological aspects are the fundamental science to manage nematodes.

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