Technical Bulletin No. 76

Root Knot Nematode: A Threat to Vegetable Production and its Management

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IIVR Technical Bulletin No. 76

Printed : December 2017, 500 Copies

Correct Citation: **Manjunatha T. Gowda, A.B. Rai and B. Singh** 2017. Root Knot Nematode: A Threat to Vegetable Production and its Management. IIVR Technical Bulletin No. 76, IIVR, Varanasi, pp. 32

Published by : **Dr. B. Singh**
Director,
ICAR- Indian Institute of Vegetable Research, Varanasi

Printed at : Army Printing Press, 33, Nehru Road, Sadar, Cantt, Lucknow-226 002, Ph. : 0522-2481164
E-mail: armyprintingpress@gmail.com

PREFACE

Vegetables are rich source of micro nutrients such as vitamins, minerals and antioxidants for balanced human diet. They play a prime role in Indian agriculture by ensuring nutritional and livelihood security. The diverse agro climatic conditions provide an opportunity to grow vegetables throughout the year. High yield, nutritional richness, ability to generate employment, annual domestic demand, health consciousness and urbanization are the important catalysts for attracting farmers to vegetable cultivation and fueling its growth in the country. Globally India ranks second with a production 176.17 million tonnes of vegetables covering 10.29 million hectares area with an average productivity of 17 .11t/ha (NHB 2016-17 estimated). However, there is a remarkable growth in vegetable area coverage and production from the last decade, nevertheless the current level of vegetable productivity is quite low in comparison with the other leading vegetable producing countries due to several constraints. Biotic stresses are one of the major constraints. Among them plant parasitic nematodes considered as one among the major pests in the vegetable ecosystem. In India, an average annual loss 19.6% has been estimated due to plant parasitic nematodes. However, in protected cultivation, an overall average annual yield loss in major horticultural crops due to nematodes goes up to 60%. Among plant parasitic nematodes, root knot nematodes (*Meloidogyne* spp.) is the most frequently observed and economic damaging genera in vegetable ecosystem.

In this endeavor, useful information on management of root knot nematodes infesting vegetable crops has been generated at Indian Institute of Vegetable Research, Varanasi and other national research institutions. However, this information is scattered in various documents and not readily available in a single publication. In view of this, efforts have been made here to compile available information in a systemic manner and present in the form of a bulletin entitled ***"Root Knot Nematode: A Threat to Vegetable Production and its Management"***. The information is presented with series of appropriate chapters that contain illustrations and descriptions of root knot nematodes and their damage symptoms. The concept of Integrated Nematode Management (INM) with its components comprising cultural practices, biological control, physical methods and chemical methods with appropriate dose, and time of application have been presented for the management of root knot nematodes in vegetable ecosystem.

The help rendered by the scientists of crop protection division in the preparation of this manuscript is thankfully acknowledged. We hope that this bulletin will be highly useful to the farmers, extension workers, state officials, academicians, students and researchers.

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1. MAJOR PLANT PARASITIC NEMATODES IN VEGETABLE ECOSYSTEM

Nematodes are soft-bodied animals generally called as round worms or eel worms or thread worms. The word "nematode" derives from a Greek word "nema" means "thread". They belonged to the phylum "Nematoda" which is extremely diverse and ubiquitous in nature. Globally species richness is estimated between 500,000 and 1 million and among them more than 28,000 nematode species have been described. Nematodes can be found in fresh water, marine and terrestrial environments. They are free-living as well as parasitic to plants and animals including human beings. Among them, plant parasitic nematodes are microscopic in size ranging from 0.5 to 2.0 mm in length and generally attack on roots, stem, foliage and flowers of plants. They possess all the organs similar to higher animals, but lack of circulatory and respiratory systems. The presence of a characteristic needle-like stylet is the key diagnostic feature for differentiating plant parasitic nematodes from all other types of nematodes. At present, about 4100 species of plant parasitic nematodes have been described. Globally, their distribution varies greatly. Among them, some are cosmopolitan, and some species restricted in particular geographical condition or some are highly host specific.

1.1 MAJOR TYPES OF PLANT PARASITIC NEMATODES BASED ON FEEDING STRATEGIES

Based on mode of feeding, plant parasitic nematodes are classified into ectoparasites, semi- endoparasites, endoparasites and foliar nematodes. Ectoparasitic nematodes remain outside of the plant and use its stylet to feed from the cells without entering into roots. Ectoparasites are most commonly observed plant parasitic nematode species under field condition, but they are considered less damaging species, for example, stunt nematode (*Tylenchorhynchus* spp.) lance nematode (*Hoplolaimus* spp.) and spiral nematode (*Helicotylenchus* spp.). Semi-endoparasitic nematodes are able to partially penetrate the plant roots and feed cell contents by developing permanent feeding cells or syncytia on plant roots, for example, reniform nematode (*Rotylenchulus reniformis*); citrus nematode (*Tylenchulus semipenetrans*). Endoparasitic nematodes enter the roots completely. They are classified in to two types a). Migratory endoparasites which migrate through root tissues by destructively feeding on plant cells, for example, lesion nematode (*Pratylenchus* spp.) and burrowing nematode (*Radopholus similis*); b). Sedentary endoparasitic nematodes are the most damaging plant parasitic nematodes genera in all over the world. They establish within the roots by developing specialized feeding sites for example cyst nematodes (*Heterodera* and *Globodera*) and the root-knot nematodes (*Meloidogyne* spp.). Foliar nematodes normally feed above-ground plant parts such as stem, leaves, inflorescence for example Ufra nematode (*Ditylenchus angustus*) and white tip nematode (*Aphelenchoides besseyi)* on rice.

1.2 ECONOMIC IMPORTANCE

Plant parasitic nematodes are of major significance inflicting substantial damage to agriculture. In the tropical and sub-tropical climates, yield losses attributable to nematodes were estimated at 14.6% in the developing countries compared to 8.8% in developed countries with an average of 12.3%. In India, the annual estimated crop losses due to major plant parasitic nematodes are estimated to the tune of Rs. 242.1 billion.

In vegetable cultivation, plant parasitic nematodes are considered as one among the major pests. In India, an average annual loss 19.6% has been estimated due to plant parasitic nematodes. However, in protected cultivation, an overall average annual yield loss in major horticultural crops due to nematodes goes up to 60%. Among plant parasitic nematodes, root knot nematode (*Meloidogyne* spp.) is the most frequently observed and key damaging plant-parasitic nematode genera in vegetable ecosystem (Table1).

Table1: Major plant parasitic nematodes in vegetable ecosystem at Research Farm, ICAR-IIVR, Varanasi

Plant parasitic nematode species	Absolute density	Relative density	Absolute frequency	Relative frequency	Prominence value	Relative Prominence value
Meloidogyne incognita, M. javanica	284	42.02	87.45	28.74	2655.83	24.35
Rotylenchulus reniformis	149	22.04	48.24	15.85	1034.82	18.08
Hoplolaimus spp.	97	14.35	66.67	21.90	792.00	21.26
Tylenchorhynchus spp.	100	14.80	72.55	23.84	851.75	22.18
Helicotylenchus spp.	46	6.80	29.41	9.67	249.47	14.12

2. ROOT KNOT NEMATODES IN VEGETABLE CROPS

Root knot nematodes are sedentary obligate endoparasites. They are distributed worldwide over a wide range of geographical conditions, preferably more prevalent in tropical and sub-tropical climatic zones and are considered to be a number one nematode problem of agricultural crops in most developing nations. Berkeley (1855) first reported the root knot disease in glasshouse grown cucumbers in England and he named this nematode as "vibrios". Subsequently, many researchers identified and described many species. Chitwood (1949) accommodated root knot nematodes under the genus *Meloidogyne* Goeldi, 1982. The genus name *Meloidogyne* was derived from Greek words which mean "apple shaped female". This cosmopolitan pest recognized its significance in global food production during later phase of 20th century and drew attention towards study on various aspects like distribution, biology, ecology, crops loss and management under International *Meloidogyne* Project (1975- 1984).

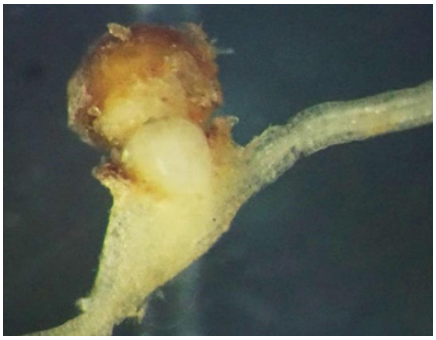
In India, for the first time Barber (1901) reported root knot nematode from tea plantation, Kerala and this is to be believed as first plant parasitic nematode reported from India. Subsequently Ayyar (1926) and many others reported infestation of root knot nematodes on number of vegetable crops.

Globally, there are 101 described species in the genus. Among identified *Meloidogyne* spp. the four major species i.e., *M. incognita* (Kofoid and White 1919) and *M. javanica* (Treub 1885) *M. arenaria* (Neal, 1889) and *M. hapla* (Chitwood, 1949) reported to cause vast economic crop damage. According to Trudgill and Blok, 2001 *M. incognita* is the single most crops damaging pathogen in the world which is responsible for $100 billion annual economic loss globally. Considerably, this polyphagous pest reported to cause an average 10% yield losses in vegetables crops and in highly susceptible crops such as tomato, eggplant and melons it is reported to cause 30% of yield loss.

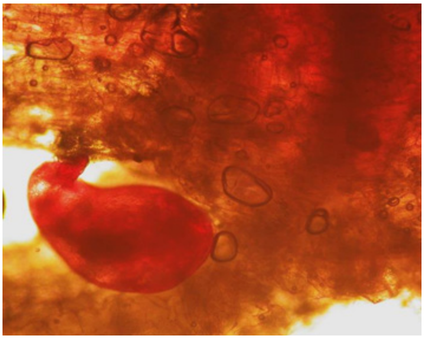
In India, fourteen species of root knot nematodes are recorded. Among them, *M. incognita* and *M. javanica* are widely distributed in different parts of the country. In addition to direct injury and migration, *Meloidogyne* acts as predisposing and facilitating agent for the entry of soil borne fungal and bacterial pathogens.

2.1 PARASITISM AND LIFE CYCLE

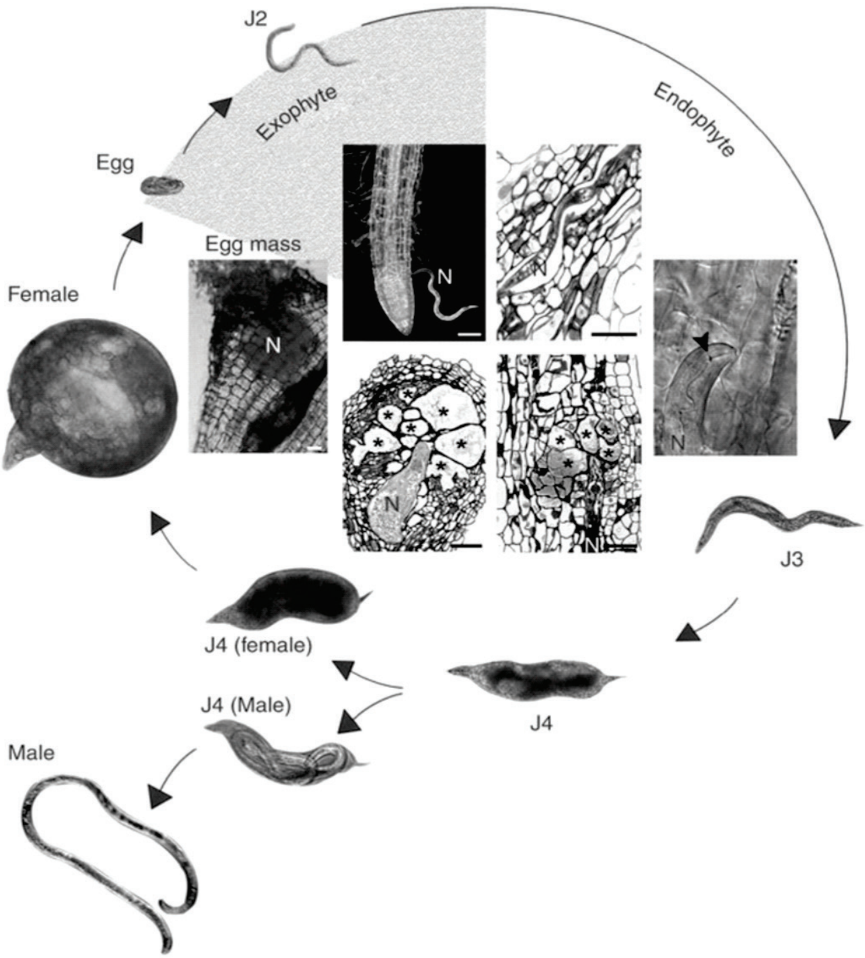
Female lays 400-500 eggs into gelatinous matrix generally protrude out of the host or some time embedded in it. Gelatinous matrix is glycoprotein complex produced from female rectal glands; act as protective agent against environmental extremes and predation. An egg undergoes embryogenesis and proceeds to first stage juvenile which moults to infective second stage juvenile. The microscopic motile second stage infective juvenile moves freely in soil in search of suitable host. However, juveniles able to survive without hosts for several months by utilizing its stored reserved food i.e. glycogen and glycolipid. When they find host, penetrate through root tips and feeds on epidermal cells. Cell contents get liquefied and semidigested through its oesophageal secretion containing hydrolyting enzymes. These nematode enzymes also induce excessive conversion of tryptophan into indole acetic acid resulting in enlargement and coalescing of pericycle cells leads to development of multinucleate giant cells. In addition cortical parenchymatous tissue in around giant cells undergo excessive multiplication leads to development of tiny swellings or galls on the roots and several of these galls merge in to big multiple galls. Giant cells serve as the permanent food source for nematode development and reproduction. Post infection, second stage juveniles continuously feed for several weeks (2-3 weeks optimally) and undergo series of three moults in quick succession and further majority juveniles develop into pyriform sedentary females. The total duration of life cycle is 3 to 4 weeks.



Infected female with egg mass



Root knot nematode female



Diagrammatic representation of root knot nematodes life cycle (Source: Abad et al., 2008)

2.2 HOST RANGE

One or more species of root knot nematodes are known to infest nearly every crop responsible for the supply of world's food, fruits, vegetables, plantations, spices, fibre, resins and ornamentals crops. They are also reported to infest and reproduce nearly on 226 weed species belonging to 43 botanical families.

Among fourteen species present in India, *M. incognita* and *M. javanica* have the widest host range infecting more than 232 and 114 genera of plants respectively. The most preferred hosts of root knot nematodes are vegetables, pulses, fibre crops, fruits, ornamentals, medicinal and aromatic plants and other important cash and plantation crops. *M. arenaria* is known to attack on 15 genera of plants including vegetables and is serious problem in groundnut crop. In case of *M. hapla* which is limited to cooler regions and host range covers about 9 genera in India.

2.3 PHYSIOLOGICAL RACES OF ROOT KNOT NEMATODES

Physiological races of root knot nematodes are known to occur in four prominent species of the root knot nematodes such as *M. incognita* and *M. javanica, M. arenaria* and *M. hapla,* which can be differentiated on the basis of North Carolina host differential test (Sasser and Carter, 1985). Currently occurrence of 4 races (race 1, 2, 3 and 4) of *M. incognita*, 3 races (race 1, 2 (pepper race) and 3 (groundnut race)) of *M. javanica* and 1 race (race 2) of *M. arenaria* have been confirmed from different parts of the country. Identification of races is an essential requirement before developing nematode resistance cultivars against the target population of any root knot nematode species.

2.4 DAMAGE SYMPTOMS AND EXTENT OF DAMAGE

2.4.1. Solanaceous Vegetables (Tomato, Brinjal, Chilli)

Economic loss: Tomato- 11-35%

Brinjal- 10-42%

Chilli- 8-23%

Nematode infestation can be typically detected first in localized areas (patches) in field, nursery or kitchen gardens. Infected plant exhibits similar to mineral deficiency or drought stress symptoms such as chlorosis, yellowing, wilting with stunted growth due to slow debility in roots function for nutrient as well as water uptake and translocation.

Root knot disease in Brinjal

Root knot disease in Tomato

However, the presence of root-knot nematodes are difficult to diagnose by noticing aboveground symptoms alone, since low fertility, poor drainage and other disease causing organisms also produce similar symptoms. Below ground typical symptoms are development of numerous "root galls". Though root galls are key diagnostic feature of root knot nematode, however size of gall depends on crop, generally large size galls are produced in tomato and brinjal crops, while in case of chilli produces very small size galls with poor root growth.

2.4.2 Cucurbitaceous vegetables

Economic loss: Cucumber- 6-18%,

Bottle gourd- 21-23%

Snake gourd- 17%

Bitter gourd- 13-14%

Pumpkin- 13%

Infestation of nematodes shows in patches due to uneven distribution of nematodes. Infested plant shows stunted growth with reduced vine length and smaller leaves with yellowish color. Broad leaved cucurbits exhibit day time wilting. Poor emergence and death of young seedlings may occur in heavily infested soil. Yield level decreased due to poor and fewer fruits in plants. Typical below ground symptoms are development of larger root galls with stunted root growth.

Root knot disease in Cucurbits: A) Bitter gourd, B) Bottle gourd, C) Pumpkin, D) Musk melon

2.4. 3. Okra

Economic loss: 10-29%

As similar to other crops, young seedlings show yellowing and stunted growth in patches of field. Poor emergence and seedling growth can be observed first few weeks after sowing. Foliage starts turning yellow from older leaves, which wither away gradually. Below ground symptoms exhibit extensive galls with stunted root growth. Finally galled roots final rots and predispose for soil borne pathogens.

Extensive galls on okra roots caused by root knot nematode

2.4.4. Root crops (Carrot, Radish)

Economic loss: Carrot: 18.20%

Parasitic activity of root knot nematodes damage the growing root tip by causes forking, distorted or stunted taproot, stubbing and fasciculation (bunching) of the roots. Typically this symptom occurs within the first few weeks after seed germination. Besides forking, induce large conspicuous galls on feeder roots. Predispose to wilt pathogen leads to development of disease complex. In general, above ground symptoms such as yellowing, wilting and patchy stunted growth was commonly observed. Infected crop reduces marketability due to deformation of roots.

Forking symptoms expressed due to root knot nematode infection A) Carrot B) Radish

Yellow patchy symptoms on radish due to root knot nematode infestation

2.5 INTERACTION WITH SOIL BORNE PATHOGENS

Root knot nematode parasitism on host plants considered to be of paramount importance in facilitating hosts for the entry of soil borne fungal and bacterial pathogen. The root exudates from root knot infected plants stimulate the entry of soil borne pathogens and aggravate the problem still further leading to development of disease complex (Table 2) and causing 40-70% severe losses in vegetable crops in the country. In addition, root knot nematode also breaks the resistance in cultivars which are resistant to soil borne fungal and bacterial pathogens.

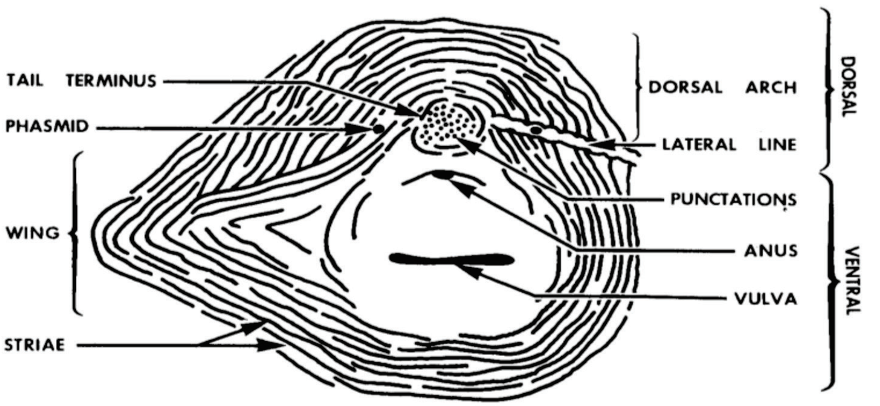
Table 2: Root knot nematodes association in the development of major disease complexes in vegetable crops

Disease complexes	Root knot Nematode spp.	Associated Pathogenic spp.	Vegetable crops	References
Damping off	*M. incognita*	*Rhizoctonia solani*	Tomato	Arya and Saxena, (1999)
Collar rot	*M. incognita*	*Sclerotium rolfsii*	Brinjal	Goswami *et al*., (1970)
Bacterial wilt	*M. incognita*	*Ralstonia (Pseudomonas) solanacearum*	Tomato	Haider *et al.,* (1989**)**
Soft rot	*M. incognita*	*Pectobacterium carotovorum subsp. carotovorum*	Carrot	Sowmya *et al.,* (2012)
Fusarium wilt	*M. incognita*	*Fusarium oxysporum f. sp. lycopersici*	Tomato	Akram and Khan, 2006
Fusarium wilt	*M. incognita*	*Fusarium oxysporum f. sp. conglutinans*	Cauliflower	Rajinikanth *et al.,* 2013
Damping- off	*M. javanica*	*Pythium debaryanum*	Tomato	Ram Nath *et al.,* 1984

3. DIAGNOSTICS OF ROOT KNOT NEMATODE

3.1 MORPHOLOGICAL IDENTIFICATION OF ROOT KNOT NEMATODES (*MELOIDOGYNE* SPP.) THROUGH PERINEAL PATTERN OF FEMALE

The cuticular markings surrounding the vulva and anus (posterior pattern or perineal pattern) of *Meloidogyne* female is used for species identification. Perineal pattern of four economically important species of root knot nematodes has described below (Eisenback, 1985).



Diagrammatic representation of perineal pattern of Meloidogyne spp.

(Source: Eisenback et al., 1981)

Meloidogyne incognita

- Striae are smooth, wavy, sometimes in a zigzag pattern.
- Lateral lines absent.
- Squarish high dorsal arch containing a distinct whorl around the tail terminus.

Meloidogyne javanica

- Striae are smooth and somewhat wavy.
- Unique distinct lateral lines or ridges run across the pattern and fading away around the tail terminus.
- Dorsal arch often low and rounded, sometimes high and squarish, frequently possessing a whorl in the tail terminus area.

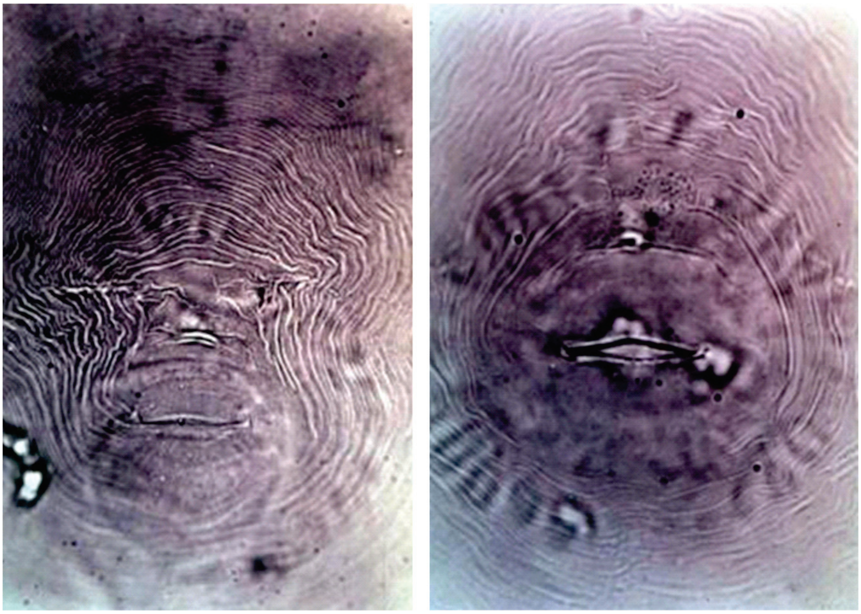
Typical perineal pattern of A) Meloidogyne incognita B) Meloidogyne javanica (Source: http://nemaplex.ucdavis.edu)

Meloidogyne arenaria

- Striae are smooth and slightly wavy, often extended laterally, forming wings on one or both lateral sides of the pattern.
- Distinctive lateral ridges are absent, but pattern marked by forked, irregular lateral fields.
- Dorsal arch low and indented near the lateral fields, forming rounded shoulders.

Meloidogyne hapla

- Striae are close, smooth and wavy, some patterns form wings on one or both lateral sides.
- Region of perineal pattern between anus and tail terminus stippled with subcuticular punctuations.
- Lateral ridges absent but the lateral fields marked by irregularities in the striae.
- Dorsal arch usually low and rounded, but may be high and squarish.



Typical perineal pattern of A) Meloidogyne arenaria B) Meloidogyne hapla (Source: http://nemaplex.ucdavis.edu)

3.2 MOLECULAR DIAGNOSTICS OF ROOT KNOT NEMATODES (*MELOIDOGYNE* SPP.)

DNA isolation

Newly hatched individual second stage infective juveniles and males are handpicked using small needle and placed in 15 µL of Worm lysis buffer (WLB) on a glass slide and cut into two pieces using stainless steel blade under a stereomicroscope. While females were picked up with tweezers and squashed with a pipette tip. The cut nematode, in 10µL WLB, is then transferred to 0.5 ml centrifuge tube containing another 10 µL of WLB. The tubes are centrifuged at 13500 rpm for 2 min, then placed at −80° C for 15 min. Mineral oil (7µL) is added to each tube and incubated at 60°C for 1 h, followed by 90° C for 10 min. The mineral oil is removed by pipette after the aqueous sample was frozen at −20°C. PCR amplifications are carried out by using rDNA or specific SCAR primers (Adam *et al*., 2007).

Table 3. Composition of worm lysis buffer (WLB) for root knot nematode DNA isolation

Worm lysis buffer (WLB)	
Potassium chloride	50 mm
Tris (pH 8.2)	10 mm
Magnesium chloride	2.5 mm
Proteinase K	60 µg/ml
NP40	0.45%
Tween 20	0.45%
Gelatine	0.01%

Table 4. Composition of PCR Reactions

PCR reactions (25 µl)	
DNA	0·5 µl
Each 10-µm primer	0·5 µl
10 × buffer	2·5 µl
50 mm $MgCl_2$	1·5 µl
200-mm of each dNTP	2·5 µl
Enzyme	2 units

Primer details

1. 5S-18S ribosome region primer (Blok *et al*., 1997)

194	TTAACTTGCCAGATCGGACG
195	TCTAATGAGCCGTACGC

Amplification size: ~ (a) *Meloidogyne incognita, M. javanica*, *M. arenaria* (720 bp), (b) *M. mayaguensis* (780 bp), (c) *M. hapla* (700 bp), (d) *M. fallax* (1600 bp) and *M. chitwoodi* (1700 bp)

Step	**˚C**	**Time**	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	50	30 secs	
Extension	72	90 secs	
Final Extension	72	7 m	

2. ***Meloidogyne incognita*** **specific SCAR,** (Zijlstra *et al*. 2000)

Finc	CTCTGCCCAATGAGCTGTCC
Rinc	CTCTGCCCTCACATTAGG

Amplification size: ~ 1200 bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	54	30 secs	
Extension	72	1 m	
Final Extension	72	7 m	

3. ***Meloidogyne incognita*** **specific SCAR primer (**Meng *et al*., 2004)

MI-F	GTGAGGATTCAGCTCCCCAG
MI-R	ACGAGGAACATACTTCTCCGTCC

Amplification size: ~ 999 bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	62	30 secs	
Extension	72	1 m	
Final Extension	72	7 m	

4. ***Meloidogyne incognita*** **specific SCAR primer (**Dong *et al*., 2001)

F	TAGGCAGTAGGTTGTCGGG
R	CAGATATCTCTGCATTGGTGC

Amplification size: ~ 1350 bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	50	30 secs	
Extension	72	1 m	
Final Extension	72	7 m	

5. *Meloidogyne incognita* specific SCAR primer (Randig *et al*., 2002)

Inc-K14-F	GGGATGTGTAAATGCTCCTG
Inc-K14-R	CCCGCTACACCCTCAACTTC

Amplification size: ~ 400 bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	64	30 secs	
Extension	72	1 m	
Final Extension	72	7 m	

6. *Meloidogyne javanica* specific SCAR primer (Zijlstra *et al*., 2000)

Fjav	GGTGCGCGATTGAACTGAGC
Rjav	CAGGCCCTTCAGTGGAACTATAC

Amplification size: ~ 720 bp

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	64	30 secs	
Extension	72	1 m	
Final Extension	72	7 m	

7. *Meloidogyne arenaria* specific SCAR primer (Zijlstra *et al*., 2000)

Far	TCGGCGATAGAGGTAAATGAC
Rar	TCGGCGATAGACACTACAAACT

Amplification size: ~ 420 bp

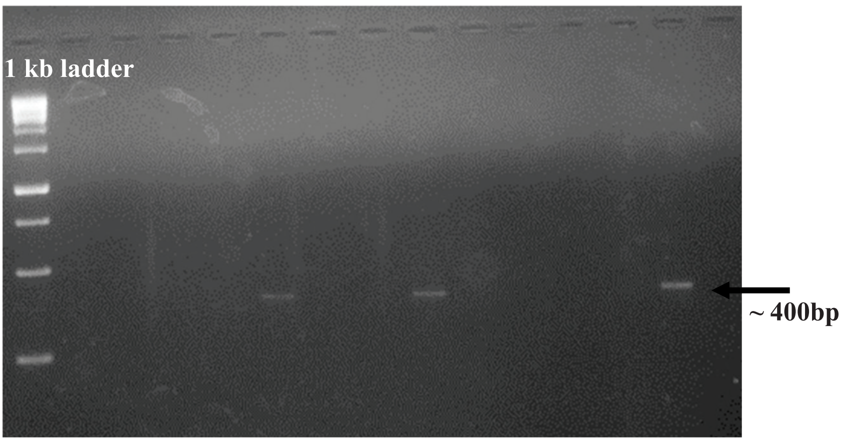
Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	61	30 secs	
Extension	72	1 m	
Final Extension	72	7 m	

8. *Meloidogyne hapla*, *M. chitwoodi* and *M. fallax* specific IGS- SCAR primer (Wishart *et al.* 2002)

JMV1	GGATGGCGTGCTTTCAAC
JMV2	TTTCCCCTTATGATGTTTACCC

Amplification size: ~ *Meloidogyne hapla* (440 bp), *M. chitwoodi* (540 bp) and *M. fallax* (670 bp)

Step	˚C	Time	
Initial Denaturation	94	2 m	
Denaturation	94	30 secs	45cycles
Annealing	50	30 secs	
Extension	72	90 secs	
Final Extension	72	7 m	

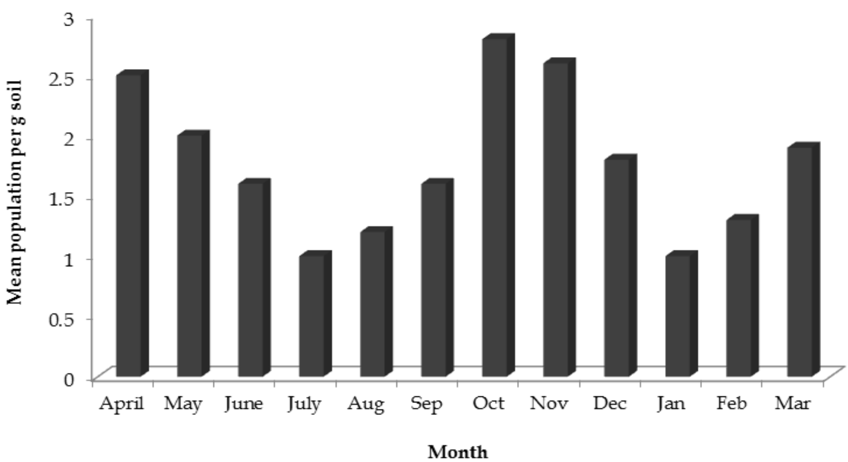


Molecular identification of Root knot nematode, Meloidogyne incognita using SCAR marker (INC-K-14F/INC-K-14R)

4. ECOLOGY AND SEASONAL DYNAMICS

In soil environment, abiotic and biotic factors largely govern the nematode behavior. Temperature and moisture are the main driving factors in soil ecosystem, however, other factors such as antagonistic flora and fauna, chemical and physical composition of soil influence the nematode behavior and their effects on the host plant. Different stages of root knot nematodes and their activities highly influenced by abiotic and biotic factors. Temperature, moisture and soil type are noted as the most important abiotic factors influencing the distribution, life cycle, survival and pathogenicity.

Generally, three prominent species of root knot nematode *M. incognita, M. arenaria* and *M. javanica* usually need relatively higher temperature for multiplication and survival than *M. hapla*. Former species require 25-30°C temperature for their growth and development. Hence these species were dominant in tropical and subtropical region of the world. However, in temperate and sub temperate regions where the temperature is around 0°C to 15°C or above, the most prevalent root knot nematode species is *M. hapla*. In northern plains of India, peak populations of root knot nematode and severe damage in vegetable crops are generally observed during September/October months and March/April months. The length of the life cycle depends on temperature which varies from 28-30 days at optimum temperature, while in the winter season it may extend > 50 days.



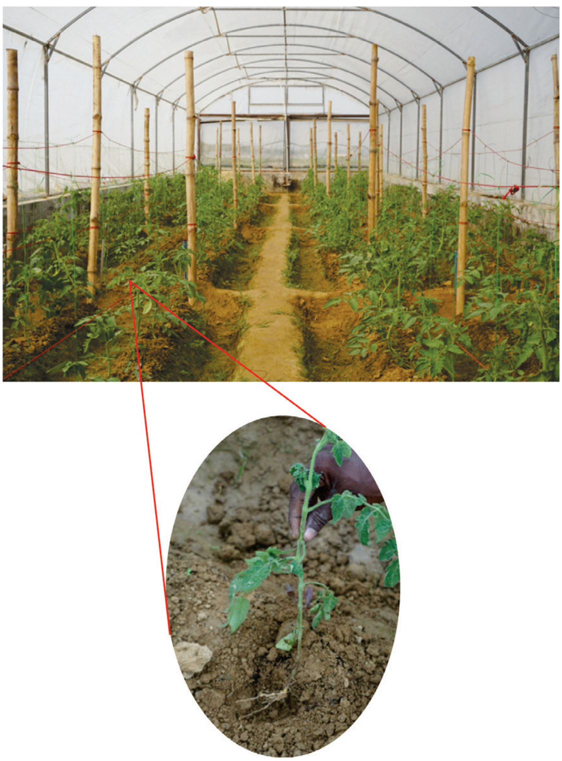
Seasonal dynamics of root knot nematode Meloidogyne incognita at Research Farm, ICAR-IIVR, Varanasi

The root knot nematodes are generally more abundant in sandy and sandy loam soils with 50% or more sand. The movement of infective juveniles and root penetration become easy and more, when the ratio of particle diameter to length of juvenile is 1:3. Nevertheless, root knot nematodes inhabit a wide variety of soils, however their damage level relatively less in clayey soil. As regards to vertical distribution, generally infective juveniles more abundant up to 20cm soil layer at field level which is highly influenced by temperature and moisture changes.

Plant parasitic nematodes require thin film of water for their survival and locomotion, which may vary among nematodes and soil types. Moisture level below (40-60%) field capacity is optimum for the activity of root knot nematode species; however they can be able to survive under low or high moisture levels.

5. MENACE OF ROOT KNOT NEMATODES IN VEGETABLE CULTIVATION UNDER PROTECTED CONDITION

Protected cultivation practices can be defined as a cropping technique wherein the micro climate (*i.e.* temperature, moisture, minerals and nutrients, light intensity and wind velocity) surrounding the plant body is controlled partially or fully as per the requirement of crops grown during period of plant growth. This practices are being followed by using green house, shade net, plastic tunnels, walk in tunnels, plant protection nets and plastic mulching. This technology helps in alleviating the demand for availability of high-quality fresh vegetable produce round the year (*i.e.* off season), for efficient use of resources and for higher productivity per unit area. However, the crops grown under protected structure are also known to be savaged by several pests and diseases. Among them, root knot nematodes (*Meloidogyne* spp.) are the most destructive and problematic plant parasitic nematode genera under protected cultivation compared to open field condition.



Tomato infected with root knot nematode under protected condition

The incidence of root knot nematodes invariably increased due to monoculturing of vegetables, use of nematode infested planting materials, contaminated soil or soil mixture used as a component of growing medium and congenial environmental conditions (i.e. higher temperature, relative humidity and use of high agronomic inputs). In comparison to open field cultivation, their proliferation rates reported to reach up to 10 to 30 fold more in protected cultivation and the population level reaches 5–6 times the threshold levels within 18–24 months. Generally average annual yield loss in major horticultural crops due to nematodes goes up to 60% under polyhouse cultivation and making the polyhouse cultivation uneconomical.

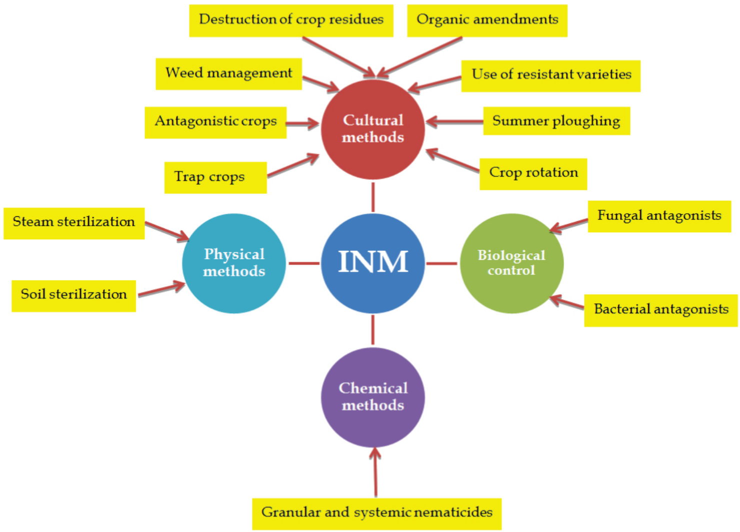
6. INTEGRATED NEMATODE MANAGEMENT

Once the root knot nematodes are established in vegetable field, it is virtually impossible to eradicate. For the effective management in infested field, it is imperative to disturb the harmonious relationship between nematode and host plant through altering soil ecosystem. However, individual techniques used for nematode management is not free from limitations. These methods are highly influenced by several biotic and abiotic factors. Hence, the concept of Integrated Nematode Management (INM) gaining importance compared to individual approach. INM is not isolated approach from Integrated Pest Management (IPM), rather a component of it. The IPM concept described by FAO, it is a pest population management system that utilizes all suitable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury. The Objectives of Integrated Nematode management (INM) is

1. To minimize environmental and health hazards.
2. Utilization of several compatible measures.
3. To maximize natural environmental resistance to plant parasitic nematodes.
4. To minimize the use of drastic control measures.
5. To increase reliance on location specific and resource compatible management strategy.
6. To minimize input costs in harmony with potential gains and maximize profit to the concerned grower.

6.1 COMPONENTS OF INTEGRATED NEMATODE MANAGEMENT

1. Cultural methods: Summer ploughing, crop rotations, antagonistic crops, trap crops, destructions of crop residues, applications of organic amendments, use of resistant varieties/hybrids/genotypes (host plant resistance).
2. Biological control: Use of nematode antagonist biocontrol agents
3. Physical methods: Soil sterilization and steam sterilization
4. Chemical methods: Need based application of granular or systemic nematicides.



Integrated nematode management

6.1.1 Cultural methods

The principles behind cultural practices are a). Prevention of new area from pest infestations b). Reduction of secondary soil inoculum once nematode is infested.

6.1.1.1 Prevention of new area from pest infestations

Root-knot nematodes are easily spread through infested soil, crop residues, vegetative propagules, human activities and irrigation water. Dissemination of root knot nematodes to newer areas is due to unawareness of nematode infestation, limitation in cleanliness standards in vegetable nurseries and also not adopting any phytosanitary measures while transporting to different parts of the country. Therefore, exclusion of infested materials is the only solitary principle to prevent newer area infestations.

6.1.1.2 Reduction of soil inoculum once nematode is infested

Once the root knot nematodes are established in vegetable field, it is virtually impossible to eradicate. Since eradication of nematode population is neither feasible nor desirable unless there are quarantine and regulatory requirements of nematode control.

Hence, nematologists advised the concept of 'living with the nematodes' by reducing inoculum level below the economic threshold level to derive maximum profits out of the management cost incurred. The following techniques may be followed to suppress root knot nematodes.

6.1.1.2.1. Summer ploughing

Two or three deep summer ploughings during the hot summer months expose the nematodes and infected tissue to solar heat and dehydration. This practice not only reduces the root knot nematode population densities, but also reduces weeds, soil borne pathogenic fungi and bacteria. Three deep summer ploughings in nematode sick field, research farm at ICAR-IIVR, Varanasi with an interval of two weeks period during May - June significantly reduced (48%) of *M. incognita* population. The efficiency of summer ploughing can be improved by soil solarisation, which helps in trapping and retaining more heat under polyethylene mulching than the direct exposure alone.

6.1.1.2.2. Destruction of crop residues

Root-knot nematodes can survive in residues of infected plants. These crop residues enhance the rate of survival of nematodes by slowing the rate of desiccation and providing mechanical protection during adverse conditions. Therefore, after harvest removal of infected plants and their destruction by burning helps in reducing inoculum densities.

6.1.1.2.3 Weed management

Weeds such as *Chenopodium album*, *Solanum nigrum*, *Tithonia rotundifolia* and other unknown weeds are known to be associated with vegetable crops. They act as alternate hosts for root knot nematodes for the perpetuation of life cycle. Thus, removal of these weeds is an essential strategy to reduce the inoculum level under field condition.

6.1.1.2.4 Crop rotation

Root knot nematodes are known to infest >3000 plant species, however, they have delineated host ranges. It is often possible to find commercially acceptable and agronomically suitable alternative crops. This practice includes certain non-hosts, graminaceous poor hosts and certain antagonistic crops for one or two years have been found effective to bring down inoculum level of nematodes. Considering to certain cropping sequences, including non-preferred hosts like sesame, mustard, wheat, maize, etc. are found to suppress the nematode population in vegetable crops. Rotation of non-host crops such as mustard, garlic, onion and cereals at least for 2 to 3 years in a suitable cropping system helps in minimizing inoculum level of the nematodes.

6.1.1.2.5 Trap crops

Trap crops are highly susceptible crops normally grown in root knot nematode infested fields. These crops which allow invasion by root knot nematodes, but do not support their development However, crops should allowed to grow over a time period to trap the infective juveniles of root knot nematodes in the roots and should be destroyed prior to reach its maturity. For example crop like *Crotalaria spectabilis*.

6.1.1.2.6 Antagonistic crops

Inter cropping or green manuring of certain crops like asparagus (*Asparagus officinalis*), mustard, and African marigold as antagonistic crops in susceptible main crop helps in suppression of root knot nematode population. Growing African marigold (*Tagetes erecta* or *Tagetes patula*) with susceptible crop helps in suppressing root knot nematode population by releasing nematotoxic compounds polyterthienyl (α-terthienyl) through root exudates respectively.

Incorporation of *Brassica* spp. such as Indian mustard (*Brassica juncea*) and rapeseed (*B. napus*) as green manures into the soil limits the reproduction of nematodes. After decomposition, they release volatile compound like isothiocyanates produced from glucosinolates, which are highly toxic to root knot nematodes. This process generally termed as bio-fumigation.

6.1.1.2.7. Organic amendments

The use of soil amendments is an age old traditional agricultural practice for enhancing soil fertility. However, they have been evaluated for their potential against major plant parasitic nematodes including root knot nematodes. Organic amendments include plant products, organic manures such as poultry, farm yard manure and vermicompost. The mechanisms of action of these amendments against plant parasitic nematodes are to (a) stimulate intense microbial activities including nematode antagonists, predators and parasites in the soil during decomposition because organic amendments act as nutrient source for these antagonists. Their survival and establishment (colonization) is considerably enhanced when applied to field; (b)Releases specific compounds after decomposition may be nematicidal (c) enhance the soil capacity to withhold nutrients, which improves plant vigor and plant tolerance to nematodes (Bridge, 1996; Oka, 2010; Akhtar and Mahmood, 1996). However, nematode management potential of an organic amendment is directly related to its nitrogen (N) content. Organic amendments with C: N ratios between 12 and 20 were highly suitable to exhibit high nematicidal activity and even to avoid phytotoxicity on crops. Organic amendments classified in two categories. They are (I) plant products (II) organic manures

I. Plant products

Numerous crops and plant species representing 57 families have been exhibited nematicidal activity against number of plant parasitic nematodes. They release of nematotoxic compounds through volatilization, exudation from roots, leaching from plants or residues, and decomposition of residues. Among identified several plants, Neem (leaf, seed kernel, seed powders, seed extracts, oil, sawdust, and oilcake) has been extensively used against control of root knot nematodes including other major plant parasitic nematodes. Particularly oil cakes are widely studied amendments and recommended in nematode management programs. Nematicidal action is due to release of chemical compounds from neem such as salanin, azadirachtin, nimbin, thionemone and various flavonoids. Besides the nematicidal effects, triterpene compounds in neem oilcake inhibit the nitrification process and increase available nitrogen for the same amount of fertilizer. In vegetable nursery, application of neem cake at 0.5 kg/m^2 area followed by solarization of the raised nursery beds with polyethylene mulch and optimum 1-2 t/ha neem cake (*Azadirachta indica*) was efficient to manage root-knot nematodes under field condition. In addition to neem cake, other available oilcakes such as castor (*Ricinus communis*), Mustard cake *(Brassica campestris),* Pongamia cake and Karanja cake have also been proved their efficacy against root knot nematode.

II. Organic manures

Application of organic manure such as poultry, farm yard manure, and vermi compost were efficient to increase plant growth parameters as well as to reduce the root knot nematode inoculum in vegetable crops. The combined application of FYM @ 10 t/ha + poultry manure @ 2.5 t/ha + bio-fertilizers (Rhizobium and Phosphate Solubilizing Bacteria) effectively reduced root knot incidence along with other plant parasitic nematodes at organic farm at ICAR-IIVR, Varanasi. Application of organic amendment ranges from 1 to 20 t/ha in vegetable cultivation. The presence of organic manure and decomposed products also stimulate increased activity of biological antagonists of nematodes.

6.1.1.2.8 Host plant resistance

Breeding and developing nematode-resistant plant cultivars are of much significance as effective and environmentally safe alternative to chemical nematicides. Some nematode resistant lines/varieties are available which can be utilized for management of root knot nematodes (Table 5).

Table 5: Root knot nematode resistant varieties / lines of different vegetable crops

Sl. No.	Crop	Resistant Variety/Resistant lines
1.	Tomato	SL-120, PNR-7,Hisar Lalit, NT-3, NT12, Pusa Hybrid-2, Arka Vardana
2.	Brinjal	Black beauty, Pant Rituraj, Banaras Giant, Rajendra Baigan, Rajendra BaiganII long, IC-90903, IC-127029, IC-122076,KS-224, IC-127040
3.	Chilli	NP-46A, Pusa Jwala, Mohini, Pusa Sadabahar, PSL-3, Surajmukhi, BSS-138, LCA-304, LCA-305, Guchheedar, Hoe-808
4.	Okra	*Abelmoschus moschatus* (two genotypes viz. IC-14O970-A, IC-203863) and *Abelmoschus angulosus* genotypes (IC-470751, IC-203834, IC-203831, IC-203833, 1C-203863)
5.	Bottle gourd	PSPL, Hoe-505, Samrat, Bogh-2

6.1.2. Biological Control

Biological control of root knot nematodes under field condition can be achieved by inoculation with effective antagonists in which high level inoculations provide immediate control (inundation strategy) or long-term effects are achieved with antagonists that can colonize the soil and remain active (inoculation strategy). In general, effective biological antagonists commercially exploited for root knot nematodes belongs fungi and bacteria. These antagonists feed or parasitize the nematodes or release secondary metabolites which are having nematicidal activity.

6.1.2.1 Fungal antagonists

Several groups of fungal antagonists are identified for the management root knot nematodes. Based on mode of action they are classified into three groups.

(I) Nematode trapping fungi

Arthrobotrys spp. and *Monacrosporium* spp. are the two fungal antagonists which trap nematodes in constricting rings and adhesive nets respectively. Their predation mechanism involves the association between a lectin secreted by the fungus and a carbohydrate secreted by the nematode cuticle. However, their predation is specific to certain nematode species and restricted availability of these antagonists in soil limits their potential use.

(II) Egg parasites

A considerable range of fungi have been reported as egg parasites and female parasites 0f root knot nematode such as *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Dactylella oviparasitica*, *Nematophthora gynophila* and *Cladosporium oxysporum*. Among them, most effective egg-parasites are *P. lilacinus* and *P. chlamydosporia* are the potential fungal antagonists successfully control by parasitizing eggs and females of root knot nematode.

(III) Toxin producing fungi

Aspergillus spp. (*Aspergillus niger, Aspergillus fumigates, Aspergillus terreus*), *Trichoderma* spp. (*Trichoderma viride, Trichoderma harzianum*) *Rhizoctonia bataticola*, *Alternaria alternata*, *Aspergillus flavus*, *Penicillium chrysogenum*, produce toxin which act as antagonists against plant parasitic nematodes. Most prominently the filamentous fungi, *Trichoderma viride, T. harzianum* strains commercially used for the management of root knot nematodes infesting vegetable crops.

6.1.2.2 Bacterial antagonists

Bacterial antagonists are grouped into two major groups, nematode parasites (*Pasteuria penetrans*) and nematode antagonistic rhizobacteria (Plant growth promoting rhizobacteria). *Pasteuria penetrans* is obligate parasite produces adhesive endospore which inhibits reproduction activity in the root knot nematode.

Plant growth promoting rhizobacteria (PGPR) are well known promising groups of microbes in agriculture due to its multiple mode of action such as competition, antibiosis, plant growth promotion and induction of systemic resistance against, plant parasitic nematodes including root knot nematodes, fungal and bacterial pathogens infecting a wide range of host plant species. Promising nematode antagonists in PGPR includes *Pseudomonas fluorescens* and *Bacillus* spp. Strains *of P. fluorescens* which produce an antibiotic DAPG (2, 4-diacetylphloroglucinol) is responsible for high nematicidal action. Hence strains producing antibiotic DAPG have been widely used for the control of root knot nematodes infecting vegetables as well as other crops. Another group belongs to *Bacillus* spp. (*Bacillus subtilis, B pumilus*) are also most promising antagonist which produce secondary toxic metabolites to suppress the population of root knot nematodes.

6.1.2.3 Application methodology

Nematicidal efficacy of these fungal and bacterial antagonists is highly influenced by several biotic and abiotic factors. Owing to enhance the biological control activity of these antagonists, various delivery mechanisms have been developed. Several promising fungal and bacterial antagonists have been identified by ICAR- IIVR and other institutes against root knot nematodes infesting various vegetable crops. The promising fungal (*Trichoderma viride*, *T. harzianum*, *Paecilomyces lilacinus, Pochonia chlamydosporia*) Vesicular Arbuscular Mycorrhiza (VAM) and bacterial antagonists (*Pseudomonas fluorescens, Bacillus subtilis)* were formulated in solid and liquid based formulation and are being used commercially for the management of root knot nematodes.

Considering significance of PGPR and their prominence on management of root knot nematode, ICAR-IIVR Varanasi has isolated several indigenous rhizobacteria from chilli and tomato rhizosphere and evaluated against root knot nematode (*Meloidogyne incognita*) juveniles. Among them, promising isolates such as *Bacillus subtilis* (CRB7, CRB9), *Bacillus marisflavi* (CRB1, CRB2) and *Alcaligenes faecalis* (CRB22) have identified and biochemically, morphologically and molecularly. These promising isolates are utilized for the management of root knot nematode in various vegetable crops under open field as well as protected cultivation.

(a) Seed treatment: Direct seeded crop such okra, cucurbits seeds are treated with fungal and bacterial antagonists @ 10 g/kg seed.

(b) Nursery treatment: For transplanted vegetable crops such as tomato, brinjal and chilli, nursery beds treated with antagonists (*Trichoderma harzianum*, *Paecilomyces lilacinus* or *Pseudomonas fluorescens*) @ 50g/m^2 area.

(c) Main field: Antagonists (*Paecilomyces lilacinus* or *Pseudomonas fluorescens*) @ 10 kg/ha enriched with 1.5 tons of FYM/ha applied on the beds 5 – 10 days before sowing seeds in okra crop effectively suppressed root knot disease incidence under field condition. Enriched respective antagonists with FYM should to be covered with mulch, optimum moisture of 25 - 30% maintained for a period of 15 days and thoroughly mixed once in week for maximum multiplication and homogenous spread of micro-organisms.

Enrichment of Nematode Antagonists

Integrated application of both the antagonists *Pseudomonas fluorescens* and fungal bio-gent, *Trichoderma harzianum* (Seed treatment (10g/kg seed and soil application of 10kg /ha with 1.5 ton FYM) effectively reduced root knot incidence in bitter gourd.

Seed treatment with *Pseudomonas fluorescens* 1% W.P. (2 x 10^8 cfu) @ 20g/kg seed and application of 5 tons of FYM enriched with 2.5 kg of each *Paecilomyces lilacinus* (2 x 10^6 cfu/g) + *Pseudomonas fluorescens* (2 x 10^8 cfu/g) have been found highly effective for reducing the root knot nematode *M. incognita* disease by increasing in marketable yield in okra.

In Brinjal, root knot nematode incidence has been effectively reduced through integrated approaches such as application of neem cake (1.5 t/ha) before 10 days of transplanting of seedlings + soil application of 10 kg/ha (talc-based formulation of antagonists *Pseudomonas fluorescens* + *Trichoderma harzianum*) enriched with farm yard manure 1.5 t/ha (FYM) at the time of transplanting.

In chilli, combined application of talc based formulations such as *Trichoderma viride* (30g/10 m^2), *Pochonia chlamydosporia* (20g/10 m^2) and neem cake (0.15 kg/10 m^2)

showed greater plant growth with a significant reduction of root knot nematode (*M. incognita)*.

In addition, other organic amendments such as neem cake (1 ton/ha), vermicompost (1ton/ha) are also used for bio-agents enrichment.

(d) On standing crop: Formulations of fungal (*Trichoderma harzianum, T. viride, Paecilomyces lilacinus*) and bacterial antagonists (*Pseudomonas fluorescens*, *Bacillus subtilis*) @ 0.5% can be drenched through manually or drip irrigation at regular interval of 30 days after sowing or transplanting.

6.1.3. Physical Methods

There are two heat based techniques will efficiently kill root knot nematodes (and other nematodes, pests or pathogens), first, steam sterilization and second soil solarization.

6.1.3.1 Steam sterilization

Steam sterilization is an effective curative physical measure that can be used to mitigate the severe incidence of root knot nematode under protected cultivation. However, this method is expensive to practice in open field condition.

6.1.3.2 Soil solarization

Soil solarization is a method of heating moist soil by covering it with transparent plastic sheets to trap solar radiation during hottest period or summer season of the year. This is technology of thermal disinfestations. Linear Low Density Polyethylene (LLDPE) clear films were efficient to manage root knot nematode incidence. The principle involved in this mechanism is (1) accumulation of heat due to transmission of short wave solar radiation and prevents loss of long wave radiation in solarized soil; (2) increase in temperature due to greenhouse effect; (3) soil moisture helps in solarization process by conducting heat energy; (4) increase in microbial and physico-chemical reactions in the soil resulting in to accumulations of gases, some being toxic pathogens and others acting as a nutrient source or induce resistance to subsequent crop and (5) prolonged exposure to higher temperature resulting in increased mortality of nematodes and also making them susceptible to antagonists.

6.1.4 Chemical Control

Judicious or need based application of nematicides is recommended as in case of highly susceptible crops and high value cash crops or for early protection of tender stages of the plant such as seed or seedling treatments in nursery bed applications**.** Carbofuran 3G and Carbosulfan 25 EC are the two carbamate group chemicals are utilized as nematicides.

- Application of Carbofuran 3G @ 0.3 g a.i. /m^2 area of nursery bed.

- Bare root treatment of seedlings with carbosulfan 25 EC @ 2 ml/litre during transplanting crops.
- Seed dressing of directly-seeded crop like okra and cucurbits with carbosulfan 25 DS @ 3% a.i. (w/w) effectively manage root knot nematode incidence in vegetable crops.
- Seed treatment with carbosulfan (25 EC) at 0.1% for overnight or root dipping 0.05% for 6 hours in cucurbitaceous crops.
- Application of Carbofuran 3G @ 1 kg a.i/ha is recommended to nematode infested vegetable crops under field condition.

7. GRAFTING TECHNOLOGY FOR ROOT KNOT NEMATODE MANAGEMENT

Grafting is an asexual, vegetative plant propagation technique which is done by connecting the root segment called "rootstock" with the shoot segment called "scion". It is practiced mainly in perennials such as fruit trees, some forest trees and ornamental plants. From recent past, grafting is also followed in vegetables especially cucurbitaceous and solanaceous crops targeting to mitigate physiological and pathological stress.

In this endeavor, ICAR- IIVR Varanasi has developed grafting technology to mitigate the problem of root knot nematode infesting vegetable crops. In this endeavour, *Solanum torvum* wild brinjal germplasm identified as resistant against root knot nematode was used as root stock to graft with scions of promising tomato varieties cv. Kashi Aman, Kashi Vishesh and Hissar Lalit. Grafted plants were compatible between root stock and scion and also showed significant resistance against root knot nematode by reducing soil population, reproduction and gall index. Thus grafting technology is a new arena of research to identify resistant root stocks and root stock breeding from vegetable germplasms to mitigate the root knot nematode incidence in vegetable cultivation should be emphasized.

Solanum torvum

Grafted Hissar Lalit with Solanum torvum

Grafted Kashi Aman with Solanum torvum

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