

# NEMATODE PESTS OF CROPS IN KERALA – an overview

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ALL INDIA CO-ORDINATED RESEARCH PROJECT  
ON PLANT PARASITIC NEMATODES WITH INTEGRATED  
APPROACH FOR THEIR CONTROL

VELLAYANI CENTRE - 695522



KERALA AGRICULTURAL UNIVERSITY

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NEMATODE PESTS OF CROPS IN KERALA – An overview

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## FOREWORD

Plant parasitic nematodes are hidden enemies of crops and present unique challenges to Agricultural Research. They cause serious reduction in growth and yield in a wide range of crop plants. They often exhibit non-specific and mis-diagnosed symptoms. Nematode population themselves are comparatively difficult to be identified and quantified. The Science of Phytonematology is only four decades old in India. Importance of nematodes as limiting factor in successfully growing crops was first realized when Ayyar (1933) reported root-knot nematode in vegetables from the southern part of the country. Later, Jones (1961) detected presence of golden nematode of potato, *Globodera rostochinensis* in Nilgiris as a pest of great economic importance. Siddiqui (1961) reported the citrus nematode, *Tylenchulus semipenetrans* for the first time in India. Another important plant parasite, the burrowing nematode, *Radopholus similis* was described on banana from Kerala by Nair *et al.*, (1966). Subsequent information on other important nematodes in various crops paved the way for starting research in the newly created departments/units at the various Agricultural Universities/ICAR Institutes.

Organised research on nematodes infesting agricultural crops started in Kerala in 1965 when a scheme to establish a Nematology laboratory was initiated at the College of Agriculture, Vellayani. Awareness about plant parasitic nematodes was created after the discovery of burrowing nematode, *R. similis* on banana in 1966. The root-knot nematode, *Meloidogyne incognita* infesting bhindi, brinjal, tomato, gourds and other vegetables, sugarcane, pulses, banana, black pepper and cardamom; the burrowing nematode, *R. similis* infesting banana, black pepper, coconut, arecanut, ginger, cardamom and lemongrass; the root lesion nematode, *Pratylenchus* spp infesting banana, rice, sugarcane and ginger; the spiral nematode, *Helicotylenchus* spp infesting brinjal, bhindi, banana, black pepper and ginger; the rice-root nematode, *Hirschmaniella oryzae* infesting rice; the lance nematode, *Hoplolaimus indicus* infesting sugarcane; the citrus nematode, *T. semipenetrans* infesting citrus and cyst nematode *Heterodera orydicola* infesting rice and banana are a few of the important nematodes prevalent in the State. Research in plant nematology was initiated at Central Plantation Crops Research Institute (CPCRI), Kayamkulam with the establishment of a separate Division of Nematology in 1972 and at CPCRI, Kasargod in 1975. Subsequently, a section of Nematology was established at National Research Centre for Spices, Calicut in 1986 later reorganized as Indiar Institute of Spices Research, Calicut in 1995.

Several noxious nematode pests were identified and their extent of damage was assessed and viable management practices formulated. The Vellayani center of the All India Co-ordinated Research Project. (AICRP) on plant parasitic nematodes with integrated approach for their control was started during June 1977. The project is operational at Nematology section of the Department of Agricultural Entomology, College of Agriculture, Vellayani under the Kerala Agricultural University. Later in addition to AICRP, research work on location specific problems was taken up under National Agricultural Research Projects with its Regional Station at Vellayani during 1982. In view of the alarming spread of the rice cyst nematode in the State, an ICAR adhoc project on "Cyst nematode, *H. orydicola*, infesting rice in Kerala" was taken up in 1985.

Later focus was on biological control of root-knot nematode infesting black pepper. A STED (Science, Technology and Environment Department) project on "Potentials of biocontrol agents for management of root-knot nematode in vegetables" was taken up during 1991-93. During 1992-94 another STED funded project "Awareness on the infestation of nematodes on medicinal and aromatic plants" was taken up. An ICAR adhoc project was formulated and implemented in 2001-2004 for investigations on nematode-plant association and their management. An NATP was taken up to chalk out technologies for mass production of bioagents of nematodes and their revalidation in farmers' field during 2001-2004.

Significant findings of the various studies undertaken on plant parasitic nematode and their management have been judiciously compiled in this publication. I congratulate and compliment Dr. M.S.Sheela and her team of talented scientists for their earnest effort to bring out this compilation and hope that the publication will provide valuable information to research and extension workers, students and farmers.



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18th October 2005  
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## PREFACE

Nematodes are an important group of crop pests requiring concerted efforts on their management. The establishment of the All India Co-ordinated Research Project accelerated researches on plant parasitic nematodes in Kerala. The painstaking efforts of nematologists of Kerala Agricultural University, ICAR institutes and other research organizations of the State led to identification of several nematode problems of crop plants, establishment of pathogenicity of key nematode pests and development of effective management strategies. During the last decade, there has been a re-orientation of management strategies for nematodes with a shift towards eco-friendly and sustainable techniques. The Nematologists of the State took the challenge and made significant contributions in this direction. An attempt has been made to compile the information generated in this publication entitled "Nematode pests of Crops in Kerala - An Overview".

The compilation has been divided into nine chapters devoted to the nematode problems of crops such as rice, vegetables, spices, coconut and arecanut, tuber crops, fruit crops, medicinal, aromatic and ornamental plants and entomopathogenic nematodes. It is a synthesis of the results of the investigations carried out in the Kerala Agricultural University, the Central Plantation Crops Research Institute, the Central Tuber Crops Research Institute and the Indian Institute of Spices Research.

We extend our sincere thanks to the contributors for their earnest effort and prompt response. We hope the information contained in the publication will serve to catalyse further organized research in nematology and resultant transfer of technology in the years ahead.

The authors express their deep sense of gratitude to:

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– EDITORS

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# REFLECTIONS ON NEMATOLOGICAL RESEARCH IN KERALA

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Plant parasitic nematodes constitute an important group of pests in agro-ecosystems. The existence of this wonder group among animals was known even from antiquity. India's ancient history (Ray, 1986, 1992) and early Chinese literature bear testimony to this. Interestingly, most of the nematodes referred to in early history were the relatively large vertebrate parasites. Still, a few reports indicated that phytonematodes too were known in the early days. The ancient Chinese symbol for a soybean root-infesting organism resembled an adult soybean cyst nematode suggesting awareness of plant parasitic nematodes (Noel, 1992). To quote Thorne (1961), the statement "sowed cockle reaped no corn" in Shakespeare's "Love's Labour Lost" in 1594 was possibly the first record of a plant parasitic nematode. Needham later identified the parasite as the wheat gall nematode, *Anguina tritici* in 1743.

Despite these early references, as a branch of science, nematology began only in the seventeenth century when compound microscope was discovered and Petrus Borellus looked with utter amazement on the 'little serpents' (*Anguillula*) in table vinegar. Subsequent reports of *Vibrio agrostis* parasitizing seeds of bent grass (Steinbuch, 1799), *Vibrio graminis* causing galls on grass leaves (Hardy, 1850), observations of vibrios in galls of a glass house grown cucumber (Berkeley, 1855) and *Anguillula dipsaci* damaging teasel (Kuhn, 1857) marked the beginning of a new branch of science

globally. Report of a serious disease of sugarbeet in Germany caused by *Heterodera schachtii* in 1859 was yet another milestone in the history of nematology. Subsequent dedication and hard work of scientists like Bastian, Butschli, Orley, de Man, Filipjev and Cobb laid strong foundations for the science of nematology through their major publications containing the description, fine illustrations and well attempted classification of Tylenchids and other nematodes. In the second half of the twentieth century, rapid strides were made in the study of nematodes and nematology bloomed into a full fledged discipline of science with diversified fields of research, perfected techniques, authoritative text books and well defined academic programmes.

The early developments in phytonematology were mainly confined to the temperate regions. In the tropics, the science began with the description of nematodes from Oceania during the late nineteenth and beginning of the twentieth century. Cobb (1891) reported nearly 30 species of nematodes from rhizosphere and plant tissues of banana grown in Fiji and described new species like *Radopholus similis* and *Helicotylenchus multicinctus*. Treub (1885) and Van Breda de Haan (1902) identified *Meloidogyne javanica* and *Hirschmanniella oryzae* from Java, Indonesia. Nowell (1919, 1920) established the etiology of the red ring disease of coconuts in the Caribbean islands. Another finding in the early part of the

century, which had a profound effect on nematology, was the recovery of *Rotylenchulus reniformis* from the pineapple fields of Hawaii in 1935 (Luc *et al.*, 1993). This led to the development of an effective nematocidal soil fumigant in the early 1940's. Subsequently, nematology laboratories were established in many subtropical and tropical countries like South Africa and India and work on nematodes gained momentum.

### Nematology in India

Origin of phytonematology in India can be traced back to the report of Barber, the then Economic Botanist working at Coimbatore on root-knot nematode infesting tea in South India in 1901. Butler (1906) identified root-knot nematode attack on black pepper in Kerala. Further, Dastur reported a disease of rice caused by *Ditylenchus angustus* in 1919. Reports of Ayyar (1926, 1933 and 1934) on *Meloidogyne* spp. infesting vegetables and other crops, Dastur (1936) on *Aphelenchoides besseyi* on rice and Jones (1961) on the golden nematode of potato, *Heterodera (Globodera) rostochiensis* from Nilgiris were other remarkable landmarks in the history of nematology in the country (Seshadri, 1986).

In spite of the early observations, organized research on plant parasitic nematodes started only in the early sixties. Several important developments led to the expansion of the science in India. Among these, the pioneering work on nematode survey and taxonomy by workers at Aligarh, Hyderabad and New Delhi, like Siddiqi, Jairajpuri, Das and Khan during 1959-65 not only laid sound and stable foundation for the growth and development of nematology in the country but also brought Indian nematology on the international map. Till date, 640 species of Tylenchids, 78 of Aphelenchids, 72 of Longidorids and 8 of Trichodorids have been

recorded (Bajaj, 1999). Reports of nematode damage to a variety of crops made by workers from time to time and the increasing realization on the role of the nematodes in limiting agricultural production too contributed to the development of nematological studies. Twenty-three species belonging to major genera like *Meloidogyne*, *Radopholus*, *Ditylenchus*, *Heterodera*, *Rotylenchus*, *Hoplolaimus* and *Helicotylenchus* were identified as important pests, causing 7.2 to 100 per cent loss in several cash and life sustaining crops in India (Patil *et al.*, 1999).

Organization of the International Nematology Course at IARI in 1964 and the South East Asian Postgraduate Nematology Courses between 1967 and 1979 and initiation of nematology teaching in the State Agricultural Universities during the same period laid the foundation for nematological teaching in India. Establishment of the Nematological Society of India in 1969 and the starting of publication of the Indian Journal of Nematology together with the organization of a series of national symposia were instrumental in creating awareness on the relevance of the subject. The establishment of the AICRP on nematode pests of crops and their control at 14 centres in 1977 strengthened the research activities in the country. Further, nematological units were established in several traditional and agricultural universities and ICAR institutes and the institutions made significant advances in basic and applied nematology.

Now, when the nematological research in the country is more than a century old, the science has come a long way from its initial stage of hesitancy. Today, this branch of agricultural discipline stands at the threshold of a mega change, gearing to reap benefit from the molecular and computer oriented hi-tech research in tune with the present day trend.



## Nematology in Kerala

The events that led to the origin of nematology in India marked the beginning of the science in Kerala too. Reports of root-knot nematode infesting tea (Barber, 1901) in Devala and pepper (Butler, 1906) in Wynad paved the way for concerted research on the pest. However, progress of nematological investigations in the State was slow for the next fifty years as has been in other parts of the country.

The establishment of the Agricultural College and Research Institute at Vellayani in August 1955 along with the report of F.G. W. Jones in 1961 on the occurrence of *G.rostochienis* on potato from Nilgiris kindled fresh enthusiasm in the study of nematodes. In 1964, late Dr. M.R.G.K. Nair, Professor and Head, Department of Agricultural Entomology, College of Agriculture, Vellayani and Dr. K. Mathan, Principal Scientist, Central Plantation Crops Research Institute (CPCRI), Kayamkulam attended the First International Nematology course at I.A.R.I., New Delhi. Subsequent report of the burrowing nematode, *R. similis* on banana by Nair and his team in 1966 had a far reaching effect impact.

### Major nematode pests

An array of nematodes has been recorded from the rhizosphere of various crops in Kerala since the first report of the root-knot nematode. Despite the occurrence of several species in appreciable densities, only a few have emerged as important pests in the State. These include the root-knot nematodes, the burrowing nematode, the rice root nematode, the cyst nematodes, the reniform nematode and the lesion nematode.

### Root-knot nematodes

The root-knot nematode comprising of

more than 60 species is universally acclaimed as the most important nematode pest of crops. Though more than 11 species are prevalent in India, *M. incognita* and *M. javanica* have the widest host range. In Kerala, *M. incognita* is wide spread and infests crops like vegetables (okra, brinjal, tomato, gourds, chilli and amaranthus), pulses, plantation crops (tea, cardamom, pepper, ginger and turmeric), tuber crops (cassava, sweet potato, colocasia, amorphophallus, coleus and yams like *D. esculenta*, *D. alata* and *D. rotundata*), ornamental plants (jasmine, anthurium and orchids) and medicinal plants (*Piper longum*, *Kaempferia galanga*, *Plumbago rosea*, *Ocimum* spp., *Rauwolfia serpentina* etc.). Besides *M. incognita*, *M. javanica* is known to infest cardamom and pepper. Recently, *M. piperi* and *M. graminicola* have been reported to infest black pepper and rice, respectively.

Besides the direct damage caused to crops, the nematode infestation augments several diseases of plants. *M. incognita* is seen associated with the slow wilt disease of pepper and rhizome rot disease of ginger. The nematode also predisposes cardamom plants to *Rhizoctonia solani* infection, which causes damping off and rhizome rot in the primary nursery. On the contrary, root-knot nematode infestation is found to be enhanced in katte affected cardamom plants.

### Burrowing nematode

The burrowing nematode *R. similis* that occurs in most of the tropical and sub-tropical regions of the world is one of the most destructive pests of crops with an extensive host range in India. The nematode causing typical lesions and rotting of roots, is a major pest of plantation crops (coconut, arecanut, and oil palm), spices (black pepper, cardamom, ginger, and turmeric), betel

vine, banana, ornamental plants (anthurium and orchids) and several medicinal plants in Kerala. It is also involved in the toppling disease of banana and slow wilt disease of pepper.

### Rice Root Nematode

The rice root nematode *Hirschmanniella* sp. is a well-recognized pest of rice. The migratory endoparasite that degenerates the normal functioning of roots is distributed throughout the world in about 46 rice growing countries including India. The species *H. oryzae* is one of the most important and early-recorded nematode pests of rice in Kerala. The nematode is seen in all the rice fields of the State occurring with a frequency distribution of 22 to 85 per cent and accounting for 5-19 per cent loss in yield. Besides the direct damage caused, infestation by the nematode predisposes rice plants to the sheath blight and sheath rot diseases.

### Cyst nematodes

The cyst forming nematodes are one of the most important and destructive nematode pests of agricultural crops all over the world. More than 75 species belonging to the genera *Heterodera*, *Globodera* and *Catodera* have been recorded globally. Even though more than 20 species are prevalent in India, only three species viz., *Heterodera oryzicola*, *G. rostochiensis*, and *G. pallida* have been observed in Kerala.

The rice cyst nematode *H. oryzicola* was reported for the first time on rice in India from Kerala. The nematode has been recorded from the paddy fields of all the districts in the state and accounts for drastic reduction in yield. Extensive cultivation of rice with more than one crop in a year coupled with presence of optimum conditions for development of the nematode in the

State presents a potential danger to rice cultivation. The banana plants raised in reclaimed paddy fields are also prone to infestation by the nematode. *G. rostochiensis* and *G. pallida* are important pests of potato.

### Reniform Nematode

The reniform nematode, *R. reniformis* first detected and described by Linford and Oliveira in 1940, ranks next to the root-knot nematodes in India. The semi-endoparasitic nematode has been recovered from the rhizosphere of vegetables like okra, brinjal and cowpea, pineapple, banana, papaya, ginger, turmeric, tuber crops, ornamentals and medicinal plants in Kerala. Yet, its infestation sets major hurdle only in the production of vegetables and cowpea in the State.

### Root Lesion Nematode

Universally, the root lesion nematode *Pratylenchus* spp. ranks high among the economically important nematodes on account of their ubiquitous distribution, wide host range, migratory endoparasitic nature and ability to cause damage in small numbers. Among 36 species reported from India, only *P. coffeae*, *P. indica*, *P. throni* and *P. zae* are of serious concern in the country.

The lesion nematode *P. coffeae* has been found to be associated with coffee, tea, ginger, turmeric, oil palm and tuber crops like cassava, sweet potato, yams, aroids and coleus in Kerala.

### Nematological investigations

Realising the importance of nematodes as pests of crops and the urgent need for formulating suitable control measures, nematology sections/departments were established at Kerala Agricultural University (KAU), Central Plantation Crops Research Institute (CPCRI), Kayamkulam, Indian

Institute of Spices Research (IISR), Kasaragod and Central Tuber Crops Research Institute (CTCRI), Trivandrum. The work carried out in these institutions accelerated the growth of nematology in the State.

### Kerala Agricultural University

Systematic research work on nematodes of agricultural crops was initiated in Kerala Agricultural University consequent to the introduction of a scheme to establish a nematology laboratory at the College of Agriculture, Vellayani in 1965. Initial research efforts in the field unveiled several nematode problems in the different crops. Decline symptoms observed in the orange plantations in the high ranges was diagnosed to be the citrus decline caused by a semi-endoparasitic nematode, *Tylenchulus semipenetrans*. Attack of root knot nematode on several vegetables, ginger, banana pepper and pulses were recorded. A new disease of rice seen in the Quilon district was diagnosed to be the symptoms of attack of the white tip nematode of rice *A. besseyii*. Another nematode infesting rice, *H. oryzae* was recorded in serious proportions from different parts of the State.

In 1969, a separate section was created in the Department of Entomology, College of Agriculture, Vellayani to streamline research activities in nematology. With the formation of the Kerala Agricultural University in 1972, research on nematodes was intensified. Random surveys were undertaken in the different districts to identify the nematodes associated with various crops. The root-knot nematode, *M. incognita* infesting bhindi, brinjal, tomato, gourds and other vegetables, sugarcane, pulses, banana, pepper, ginger and cardamom; the burrowing nematode *R. similis* infesting banana, sugarcane, ginger, coconut, pepper, coconut,

cardamom and lemongrass; the root lesion nematode *Pratylenchus* spp. infesting banana, rice, sugarcane and ginger; the spiral nematode *Helicotylenchus* spp. infesting brinjal, bhindi, banana, pepper and ginger; the rice root nematode *H. oryzae* and the cyst nematode *H. oryzicola* infesting rice and *Hoplolaimus* sp. infesting sugarcane, were some of the important nematodes identified during the period.

Increasing realization on the importance of the organism as pest of crops led to the establishment of a centre of the AICRP on Nematode Pests at Vellayani in 28.06.1977, financed by the ICAR. Implementation of the project led to an upsurge in the research activities on plant parasitic nematodes. The major objectives of the project were

- To conduct co-ordinated investigations on important plant parasitic nematodes
- To identify and map the nematode fauna with particular reference to economically important crops and to record their frequency of occurrence
- To determine yield losses at varying nematode densities
- To determine damage thresholds of economically important nematodes, population dynamics and nature of host parasite relationship
- To evaluate the germplasm collections for their degree of resistance to nematodes
- To develop economically viable and feasible methods of integrated nematode management practices

The project continues to function in the Nematology section of the College of Agriculture, Vellayani. With the introduction of the National Agricultural Research Project (NARP) at the Regional Agricultural

Research Station (Southern Region), Vellayani in 1982, location specific nematode problems were taken up with a view to assess the damage potential of nematodes in a multicropping system like homesteads and to generate suitable technologies to manage them. Detailed studies were conducted on the distribution pattern and seasonal population fluctuation of nematodes in different crop combinations and soil types (Sheela, 1997).

### Salient Achievements

#### *Crop problems*

- Rice root (*H. oryzae*) and cyst (*H. oryzicola*) nematodes were identified as important limiting factors in the cultivation of paddy. The damage potential and pathogenicity of *H. oryzicola* was established.
- A new nematode pest of rice, *M. graminicola* was reported.
- High population of root-knot and reniform nematodes were recorded from almost all vegetable fields
- Root-knot and burrowing nematodes emerged as major production constraints in banana, pepper, ginger, turmeric and aromatic (vetiver and lemongrass) and medicinal plants (*P. roseae*, *P. longum* and *K. galanga*)
- Padukutty and Pampadurnpara in Idukki district, Konni in Pathanamthitta district and Onamkutty and Kayamkulam in Alappuzha district were identified as hot spots for *H. oryzicola* of banana
- The pathogenicity and crop loss due to different nematodes in different crops were assessed
- Mode of host-pathogen relationship of different crops and important nematodes were established.

### Nematode management

- Solarisation of nursery beds was demonstrated to be a highly effective technique for controlling nematodes in transplanted crops like brinjal
- Screening programmes for the identification of sources of resistance to root-knot nematode in okra, brinjal, tomato, chilly, pepper and ginger were undertaken and varieties with some degree of tolerance were identified
- Organic amendments like oil cakes of groundnut, castor, neem and mustard; soil conditioners like sawdust, coconut husk powder, paddy husk powder, lemon grass waste, cashew shell powder and sugarcane bagasse; green leaves of mango, glyricidia, neem, clerodendron and eupatorium were identified to have either nematostatic or nematicidal properties
- Integrated nematode management strategies involving seed /nursery treatment, deep ploughing and application of granular insecticides in the main field were evolved for the control of nematode pests of okra and brinjal
- Recommendations were evolved for the control of nematodes infesting rice, okra, brinjal, cowpea, banana, ginger, pepper, coleus, amorphophallus, *P. roseae*, *K. galanga*, *P. longum*.
- Six bacterial pathogens; *Bacillus macerans*, *B. circulans*, *B. coagulans*, *B. subtilis*, *B. pumilis*, and *B. licheniformis* were isolated from root-knot nematode. These bacterial antagonists were reported for the first time from Kerala.
- Techniques for mass multiplication of *B. macerans* and *Paecilomyces lilacinus* have been standardized and formulations developed for distribution to the farmers.

- The efficacy of a native isolate of *Pseudomonas fluorescens* ( P1) in controlling root knot nematode too was established.
- Among nematode antagonistic fungi, mycorrhizal fungi like *Glomus fasciculatum*, *G. etunicatum*, *G.constrictum*, *G. mosseae*, *G. monosporum* and *Acaulospora morrowae* proved potent in controlling root knot nematode infesting cowpea, amaranthus, brinjal, pepper and ginger
- Seven nematophagous fungi viz., *Alternaria alternata*, *Drechslera tetramera*, *Trichoderma viride*, *Syncephalastrum racemosum*, *Curvularia lunata*, *P. lilacinus* and *Beauveria bassiana* were identified as highly pathogenic to the cyst nematode.
- Indigenous isolates of the entomopathogenic nematode *Heterorhabditis* sp. was extracted from paddy soils and pests from different localities

#### Major recommendations / Technologies developed

The following recommendations evolved for the control of plant parasitic nematodes were included in the State level package of practices recommendations of the Kerala Agricultural University.

#### Rice

- Nursery treatment with carbofuran @ 1kg a.i./ha and seedling root dip in 0.2% dimethoate for 6 h before transplanting

#### Okra

- Application of sawdust or paddy husk @ 500g/plant, neem leaves or eupatorium leaves @ 250g/plant in the basins three weeks prior to planting and watering daily.
- Application of neem or eupatorium leaves @15t/ha two weeks before sowing for controlling root- knot and reniform nematodes

- Seed treatment with *B.macerans* @ 3%w/w of seeds for the management of nematodes.
- Seed treatment with *B.macerans* @ 3% w/w of seeds + drenching pits with 2% solution of the formulated product of *B.macerans* 30 days using starch solution after sowing (concentrated may used as sticking agent for seed treatment) in endemic areas.

#### Brinjal

- Nursery treatment with @ 25 g/m<sup>2</sup> + drenching with 2% solution of formulated product of *B.macerans* 7 days after sowing.

#### Banana

- Paring banana suckers and dipping in hot water at 55 C for 20 minutes and application of neem cake 1kg/plant or carbofuran 0.5g a.i/ha for the control of nematodes

#### Ginger

- Application of neem cake @1t/ha at planting + carbofuran 1kg a.i/ha at 45 DAP.
- Application of carbofuran @3.3g/ m<sup>2</sup> or neem cake (200g/m<sup>2</sup>) at the time of planting and neem cake (100g/ m<sup>2</sup>) 45 DAP in endemic areas.

#### Pepper

- Application of *B. macerans* @1.2 x 10<sup>8</sup> cells / vine just before monsoon period for controlling root knot nematode.

#### Thippali (*P. longum*)

- Application of *P. fluorescens* formulated product @ 5g/pit or carbofuran @ 0.5 g/plant at the time of planting of the rooted cuttings (10g/m<sup>2</sup>).

#### Kacholam (*K. galanga*)

- Rhizome treatment with *B.macerans* formulated product @3% w/w of seed material.

- Mulching with neem or glyricidia or clerodendron @ 5kg/m<sup>2</sup> 30 days after planting.

#### Chethikoduveli (*P. roseae*)

- Application of *P. fluorescens* 10g/m<sup>2</sup> or carbofuran 3-5 g/pit.

#### Coleus

- Deep ploughing of the field in summer (30-45 cm) and crop rotation with non-preferred hosts like tapioca and sweet potato (var. Sree Bhadra) for controlling root knot nematode.

#### Research guidance/ Trainings

The Division offers both PG courses and research guidance to students on nematology. Twenty-eight M.Sc. and 4 Ph. D. students who carried P.G. research in nematology have been awarded degree. Besides, the faculty has also supervised and guided students with specialization in nematology from other universities. Apart from the PG programmes, trainings were also imparted to officers of the Department of Agriculture on management of major nematodes.

#### Publications

Research papers	: 98
Reviews	: 14
Technical bulletins	: 4
Popular articles	: 50
(Sheela 2001 , Sheela, <i>et.al.</i> , 2000)	

#### Central Plantation Crops Research Institute, Kayamkulam

The CPCRI was established in 1970 with Kasaragod as its headquarters. Research in plant nematology was initiated at its regional station at Kayamkulam, with the establishment of the Division of Nematology in October 1972. The station took the

lead in conducting research on nematode problems of plantation crops like coconut, arecanut, oil palm, cashew nut, cocoa and spices.

#### Salient Achievements

- Widespread occurrence of *R. similis* in coconut plantations of Kerala was recorded, the percentage occurrence being 12.6 in healthy areas compared to 29 in root (wilt) disease prevalent areas. Thirty-nine genera of plant parasitic nematodes were identified from the root zone of coconut. Six new species viz., *Brachydorus swarupi*, *Chronogaster spinicarpus*, *Ecphyadophora teres*, *Ecphyadophoroides leptcephalus*, *E. macrocephalus* and *Epicarinema keralense* were described. The coconut isolate of *R. similis* was found to have a wide host range.

- The threshold level of *R. similis* was determined to be one nematode in 625 cm<sup>3</sup> or 900g sandy loam soils. The nematode population survived for six months in moist soil and one month in dry soil.

- The non- involvement of the nematode in the root (wilt) disease complex was established.

- The dwarf coconut cultivars Kenthali and Klappawangi and the hybrids JG x KDY, KDY x JG, J x MDY and San Ramon x Gangabondam recorded least nematode multiplication.

- *P. lilacinus*, *P. penetrans* and species of AMF like *G. mosseae* and *Acaulospora bireticulata* were identified to have potential for biomanagement of *R. similis*. A fungal parasite *Ecatenaria vermicola* was reported from *R. similis*.

- Intercropping with *Crotalaria* was found to be effective in reducing *R. similis* population.

- Soil application of carbofuran or phenamiphos @ 2-4g a.i. per plant was effective in controlling *R. similis*
- Natural occurrence of an entomopathogenic nematode *Heterorhabditis indicus* found attacking red palm weevil, rhinoceros beetle and white grubs was reported from *R. similis*
- *R. similis* was the most important nematode pest of arecanut. The population of the nematode was more in Yellow Leaf Disease affected pockets (74%) than in disease free locations (44%)
- The cultivars Indonesia-6, Mahuva-B and Andamans-5 were identified as tolerant to nematode infestation in arecanut.

#### Research Guidance/ Trainings

Five students who worked in the nematology laboratory at CPCRI received Ph.D. degree from the University of Kerala, Trivandrum. Training was imparted to students from Netherlands and Sri Lanka and scientists from CRI, TRC of Sri Lanka. Training programmes are also being organized for nematologists in India.

#### Publications

Research papers	: 160
Reviews	: 30
Book Chapters	: 13
Popular articles	: 20

(Koshy and Sosamma, 2001)

#### Indian Institute of Spices Research, Calicut

A regional station of the Indian Council of Agricultural Research was established under CPCRI at Kasargod in 1975 for conducting research exclusively on spice crops. The regional station was upgraded to National Research Centre for Spices (NRCS)

in 1986 and again to a full-fledged institute, the Indian Institute of Spices Research (IISR) in 1995. Slow decline disease caused by *R. similis* and *M. incognita* in black pepper, root knot nematodes in cardamom, root knot and lesion nematodes in ginger and turmeric are the major nematode problems of these crops. The Nematology section under the Division of Crop Protection of the station addresses these aspects.

#### Salient Achievements

- Fourteen genera of plant parasitic nematodes which included sedentary endoparasites (*Meloidogyne* spp.), migratory endoparasites (*R. similis*, *Pratylenchus* sp.), semi-endoparasites (*Trophotylenchulus piperis*, *R. reniformis*) and ectoparasites (*Acontylus* sp., *Aphelenchus* sp., *Criconemoides* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Longidorus* sp., *Scutellonema* sp., *Tylenchorhynchus* sp., *Xiphinema* sp.) were reported to be associated with black pepper. Among these, the occurrence of the root knot nematode (*M. incognita*) was maximum (69.8%), followed by the burrowing nematode (*R. similis*, 57.5%).
- Two new species of nematodes viz., *T. piperis* and *M. piperi* infesting pepper were described.
- The role of *R. similis* and *M. incognita* in the etiology of slow wilt disease of black pepper was established
- Technique was standardized for screening black pepper germplasm for resistance to nematodes. The technique involves inoculation of rooted cuttings at 3-4 leaf stage @150 nematodes / cutting and recording root lesion index 6 months after inoculation for *R. similis* and for *M. incognita* inoculation @ 1000 second stage juveniles and recording root knot index at 4 months after inoculation.

- Among the germplasm accessions screened against *R. similis* and *M. incognita*, the wild related species *P. colubrinum* was resistant to both the nematodes while the cultivar Ottaplakal-I was resistant to *M. incognita*.
- Two nematicidal principles characterized as genkwanin and luteolin 7-o-methyl ether were isolated from the leaf extract of *P. colubrinum*
- Association of eight genera of plant parasitic nematodes namely, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Criconeoides*., *Helicotylenchus*., *Longidorus*, *Tylenchorhynchus* and *Xiphinema* was reported in ginger. *Meloidogyne* sp. was the most predominant followed by *R. reniformis*.
- Three accessions of ginger (Acc. Nos. 36, 59 and 221) were identified resistant to root-knot nematode.
- Six genera of plant parasitic nematodes namely, *Meloidogyne*, *Rotylenchulus*, *Pratylenchus*, *Criconeoides*, *Hoplolaimus* and., *Longidorus* were observed associated with turmeric, the root knot nematode being the most predominant species (78%) with high population (127/100cc soil).
- Eight accessions of turmeric (Acc. Nos. 31, 82, 84 142, 178, 182, 198 and 200) were identified resistant to root-knot nematode
- AMF like *G. mosseae*, *G. fasciculatum*, *Acaulospora laevis* and *Gigaspora margarita*, the fungi *Verticillium chlamydosporium* and *P. lilacinus* and bacteria like *P. penetrans* and *Trichoderma harzianum* were found effective in controlling nematodes of different spices.

#### Major recommendations / Technologies developed

##### Black pepper

- The high yielding cultivar, Ottaplakal-1 identified as resistant to *M. incognita* was released as Pournami for cultivation in root-knot nematode infested plantations.

- A package which includes application of neem cake @1kg/vine along with phorate 10G or carbofuran 3G @ 3g a.i /vine during May/June and again September/October was recommended for adoption for the control of plant parasitic nematodes infesting pepper.
- Recommended the use of AMF isolates in nursery mixture for producing nematode free planting materials.
- Integrated management schedule for slow decline disease was developed which included application of fungicides in combination with nematicides and biocontrol agents.

##### Cardamom

- Soil solarisation for 40-45 days as a pre-sowing treatment for controlling nematodes and other soil borne disease of nurseries.
- Incorporation of *T. harzianum* multiplied on decomposed coffee husks (7days old) at the time of sowing @2.5 kg/bed (4.5 x 1m) and three months later for controlling root knot nematodes and damping off in nurseries.
- Application of phorate (5 g a.i/bed) and 0.2% copper oxychloride (20 l/bed) two weeks after germination of seeds and again after three months for controlling root knot nematodes and damping-off in nurseries.
- Spot application of phorate @ 2.5 g a.i/ clump, twice a year during April-may and October - November for controlling root knot nematodes, thrips and root grubs in plantations.

##### Publications

Review / status paper	: 9
Research papers	: 59
Symposia/seminars/workshops	: 30
Book chapters	: 7
Popular articles	: 3
Books/bulletins	: 1
Technical reports	: 3
Extension pamphlets	: 1

(Ramana and Eapen, 2001)



## Central Tuber Crops Research Institute, Trivandrum

The Central Tuber Crops Research Institute (CTCRI) was established in the year 1963 at Thiruvananthapuram, Kerala. It is the only organization in the world dedicated solely to the research and development on tropical tuber crops viz., cassava, sweet potato, yams, aroids and minor tubers. Research on nematological problems of tuber crops was initiated in 1988.

### Salient Achievements

- *M. incognita* was found to be the most widespread nematode pest infesting cassava, sweet potato, yams, aroids and coleus. Other nematodes found were *Pratylenchus* sp., *R. reniformis*, *Hoplolaimus* sp. and *Scutellonema* sp.
- Pathogenicity of *M. incognita* on sweet potato, amorphophallus, colocasia and *D. esculenta* was established
- Accessions of cassava and sweet potato resistant to *M. incognita* were recorded.
- In colocasia, Sree Pallavi was found to be highly susceptible to the nematode whereas C9, a cultivar was highly resistant. In *D. esculenta*, De 53 was found to be highly susceptible whereas Sree Latha was found to be resistant to the nematode.
- Nematicidal efficacy of cassava amendment was established. Field wastes of cassava (leaves) and factory wastes (tuber rind) were found to be effective in suppressing nematode multiplication and increasing plant growth characteristics.
- Sree Bhadra, a high yielding short duration variety was identified as resistant trap crop. When this variety was planted in root-knot infested field the nematode population declined to below detectable level over a single cropping duration of 90-95 days.

### Publications

Research papers	: 60
Reviews	: 3
Technical bulletins	: 2
Popular articles	: 5

(Mohandas, 2002)

The study of nematodes initiated with the first report by Barber (1901) was nurtured through the dedicated and multipronged research carried out by scientists from various organizations and progressed into a full fledged scientific field in the State. Modern and fully equipped laboratories established in the nematology units catered to every aspect of nematological investigations. Significant accomplishments were achieved in fundamental and applied nematology. A host of nematode pests were identified and a range of approaches was evolved to curb them. The management options include nursery treatments (nematicides and biological antagonists), solarisation, crop rotation and use of organic amendments, botanicals, bioagents and nematicides. Integrated nematode management strategies (INM) too have been developed incorporating some of the components. Despite promising leads obtained, utilization of resistant cultivars and potent bioagents, which form the core of INM strategies, is still in the exploratory stage. With organic farming playing a pivotal role in agriculture now-a-days, it is imperative to evolve environmentally benign and economically feasible biointensive integrated nematode management (BINM) strategies suited to our farming situations. The information consolidated and technologies generated over the years could be refined with modern techniques and methodologies to achieve the target. In the emerging scenario, the futuristic research in nematology should be on the following lines

- Identification of resistant sources against nematodes of different crops and development of acceptable varieties through exploitation of biotechnology and molecular biology
- Identification of indigenous strains of bioagents, development of cost effective and easily adoptable technologies for their mass production
- Development of remunerative cropping sequences and trap crops
- Development of expert systems based on molecular tools for accurate identification of nematodes
- Promotion of farmer- participatory nematode management

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## Symptoms of nematode infestation



*M. graminicola* in rice



*M. incognita* in pepper



*R. similis* in banana



*M. incognita* in long coriander



*R. similis* in coconut

# NEMATODE PESTS OF RICE

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Rice is the most important food crop that feeds millions of people all over the world. The population of rice consumers is increasing at a faster rate of 2 per cent as opposed to 1.4 per cent overall annual rate of growth. According to one estimate, global rice production must reach 800MT from the present 599MT to meet the demand in 2005. The increased demand of rice will have to be met under several constraints. Pests and diseases constitute the major challenges to rice. Among the major pests, nematodes constitute a major threat to rice production. The association of nematodes with some pathological and physiological maladies of rice has only been appreciated recently.

Kerala is one unique state in India wherein rice is cultivated in a variety of heterogenous ecosystems. The annual rice output in the State is vulnerable to fluctuation due to infestation by several insect and nematode pests. The rice root nematode *Hirschmanniella oryzae*, the rice cyst nematode *Heterodera oryzicola* and the root-knot nematode *Meloidogyne graminicola* are the major nematode pests of rice in the State. Both *H. oryzae* and *H. oryzicola* are seen in all the rice tracts, the former being predominant and the latter, the most destructive. *M. graminicola* is of recent occurrence and has been recorded from only a few districts (KAU, 2001).

## *Hirschmanniella oryzae*

The rice root nematode is a migratory

endoparasite. Intercellular migration of the nematode in the cortex produces cavities and channels and degenerates the normal functioning of the roots. The symptoms produced due to infestation of the nematode include stunted growth, chlorosis, reduced tillering, delayed flowering and physiological disorders. The symptoms become conspicuous when heavy population of the nematode is seen. Severely infested plants show delayed tiller emergence and discolouration of older leaves (Das and Rao, 1971). Total sugars, amino acids and phenols in the plants are reduced due to infestation. At low levels of nematode infestation there is no appreciable reduction in leaf chlorophyll, starch and proteins.

A number of weeds like *Echinochloa*, *Cyperus* and *Monochoria* growing in the field serve as host for this nematode and account for build up of the nematode population.

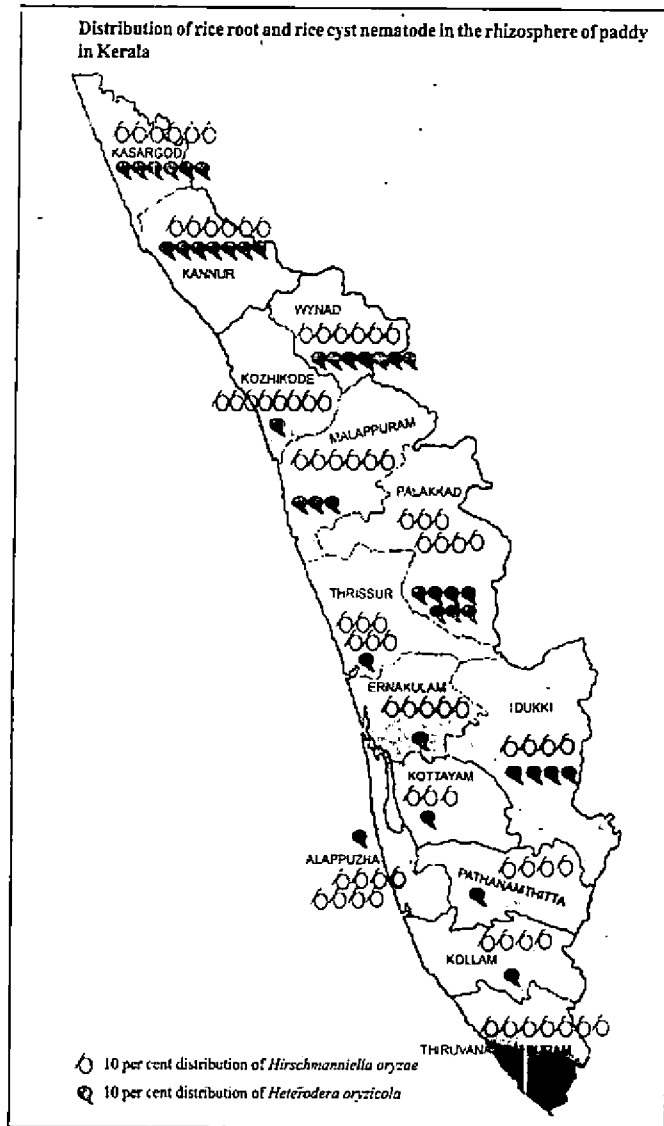
## Distribution

*H. oryzae* is widespread throughout the rice growing regions of India in varying proportions (Rao *et al.*, 1969; Rao and Israel, 1971; Roy, 1987; Sharma *et al.*, 1992). The nematode received the attention of researchers with the establishment of the role of the nematode in the incidence of a malady involving leaf drying and stem rot of rice in Bihar during 1962. Subsequently, Mathur and Prasad (1971) reported the presence of the nematode from 12 states of the country.

The nematode was also detected in the rice fields of Kerala (Venkitesan and

Charles, 1979). Consequently, regular and systematic survey of all the rice growing tracts located in various districts of the State was done from 1978. Soil and root samples were collected from 1634 different locations in Thiruvananthapuram, Kollam, Alappuzha, Pathanamthitta, Idukki, Kottayam, Ernakulam, Thrissur, Palakkad, Malappuram, Kozhikode,

Waynad, Kannur and Kasaragode districts (KAU, 1997). The results of the extensive survey revealed that rice root nematode occurred in all paddy growing tracts in the State with a percentage frequency distribution of 30 to 80. Minimum distribution was seen in Kottayam (30 per cent) and maximum in Kozhikkode and Alappuzha District (80 per cent) (Fig 1).



## Crop loss

It is estimated that *Hirschmanniella* spp. infest 58 per cent of the world's rice fields causing 25 per cent yield loss (Luc *et al.*, 1993). In India upto 81 per cent loss in yield was recorded at an initial population level of 1000 nematodes per pot (Mathur and Prasad 1972). Inoculation of 1000 or 2000 nematodes per plant adversely affected growth and yield of rice. Significant reduction in fresh shoot and root weight of rice at all inoculum levels (i.e. 250, 500, 1000 and 2000 nematodes/kg soil) were reported (Dalal, 1985).

Experiments with varying levels of *H. oryzae* have established different degrees of yield loss in Kerala. Preliminary investigations indicated that an initial population of 45 to 136 *H. oryzae* in 200g of soil in the field reduced production of tillers up to 15 per cent and yield of paddy from 14 to 19 per cent. In another trial, 6.2 to 13.8 per cent reduction in the production of tillers and 7.9 to 22.5 per cent loss in yield were observed at the same population levels. When the trial was repeated, a yield reduction of 5 to 19 per cent was obtained with same levels of population of the nematode. In the trial conducted to establish its pathogenicity, population level of 500, 250 and 50 nematodes per 200 g of soil were found to result in significant decrease in yield of grains by 53.6, 25.9 and 15.2 per cent, respectively (Hebsybai *et al.*, 1996).

Apart from the loss incurred in yield, the nematode was also observed to be associated with certain diseases of rice. A survey conducted at the State Seed Farm Adoor, Kollam district where the sheath blight disease was found to be endemic revealed the occurrence of high populations of the rice root nematode. Nematode infestation was

observed to be higher in rice plants severely affected by sheath blight indicating a positive relationship between nematode infestation and disease incidence. Inoculation of heavy population of the nematode along with sheath blight fungus to potted plants produced maximum intensity of sheath blight, indicating a synergistic relationship between the fungus and the nematode wherein one favoured the multiplication of the other (Gokulapalan, 1981).

## Management

In view of the wide spread occurrence of the nematode in the important rice growing tracts of the country, its management strategies has received most attention. Various methods like adjustment of date of planting fallow, weed control, use of various amendments application of fertilizers, rotation with non-host plants, nursery and mainfield treatment with chemicals, seed soaking and root dip in chemicals have been tried for the control of the nematode. Use of organic amendments such as mustard or neem oil cakes showed promising reduction in the population of *H. oryzae*. Nematode infested fields properly fertilized with NPK were also found to suffer less damage than the unfertilized infested field. Various chemicals and different modes of application like root dip of seedlings at transplanting and soaking of seeds have been used for the management of *Hirschmaniella* spp. Granular formulations of nematicides such as diazinon, fensulfothion, aldicarb, carbofuran phenamiphos, phorate etc. were found to reduce the nematode population both in soil and roots. (Setty and Reddy 1969 Muthukrishnan *et al.*, 1977, Chhabra and Dhaliwal 1978). In Kerala too several approaches were tried to identify a control measure suited to the local conditions.

## Chemical control

The initial attempts were mainly concentrated on chemicals. Field experiments were done to find out the effect of nursery soil treatment and seedling root dip for controlling *H. oryzae*. Rice nursery was treated with DBCP (Nemagon 60 EC @ 30L per ha) 7 days before sowing the seeds. At transplanting the seedlings were given root dip in 0.2 per cent solutions of carbofuran, monocrotophos, quinalphos, dimethoate, phosphamidon and aldicarbsulfone. Nursery treatment (DBCP) along with seedling bare root dip in phosphamidon and aldicarbsulfone (0.2 per cent) recorded maximum yield (Kuriyan *et al.*, 1980). In another field trial, nursery treatment with metham sodium @ 250 l/ha + phenamiphos 0.2 per cent seedling root dip for six hours was found very effective for controlling the nematode. Nursery treatment with carbofuran @ 1kg ai/ ha + carbofuran sulfone seedling root dip for half an hour also proved effective (Hebsy bai *et al.*, 1992).

Adaptive trials involving nursery treatment with carbofuran @ 3g ai/m<sup>2</sup> and main field application of carbofuran @ 1kg ai /ha at 40 DAT demonstrated the efficacy of the treatment in checking infestation of rice root nematode. Grain and straw yield of paddy was increased, the increase in yield of grain ranging from 5-13.75q/ha and straw yield from 9.5- 18.5 q/ha. (KAU, 2003). The package is being recommended for adoption in endemic areas of rice root nematode in the State.

Combined application of the fungicide Vitavax 1% or Fycop 4% along with the nematicide carbofuran significantly reduced the intensity of sheath blight and nematode infestation and increased the grain yield considerably.

Despite the efficacy of chemicals in controlling the nematode, there is little

indication that chemical control is economical or practical. Considering the constraints involved, cost effective methods were also explored for the control of the nematode in the State.

## Nutrition Management

Considering the fact that yield losses due to *Hirschmanniella* spp. are greater in poor soils and the possibility to reduce losses by improving nutritional status of soils (Mathur and Prasad, 1972), effectiveness of soil application of organic and inorganic fertilizers especially nitrogen on the management of rice root nematode was studied under field conditions. Nitrogen was applied in the inorganic form as ammonium sulphate and calcium ammonium sulphate and in organic form as water hyacinth to give 60kg of nitrogen per ha at planting. The results of the field trial revealed that application of water hyacinth gave maximum reduction in population of *H. oryzae* in the soil 50days after planting (34% ) and at harvest (14%) and improved the yield of paddy (KAU, 1997).

## Crop rotation

Host plant characteristics, a vital determinant of population dynamics influence the pathogenicity, density, composition and duration of activity of plant parasitic nematodes. Consequently, crop rotation with non host crops discourage the build up of noxious species beyond tolerable levels. The potential of this strategy in the management of *H.oryzae* in rice based cropping systems was also explored. Seven cropping sequences were studied and in each sequence paddy was raised during the first and second crop season. During the third crop season, other crops like daincha, green gram, groundnut, cowpea, sesamum and okra were

raised. The results indicated that rotation with different crops and fallow reduced the population of *H. oryzae*. When paddy was further raised in the second crop season, a gradual build up of the nematode population was observed. Subsequently, during the third crop season when crops like daincha, green gram, groundnut, sesamum and cowpea were raised, the nematode population was considerably reduced. Fallowing too was effective in suppressing the nematode. Paddy-paddy-okra rotation did not reduce the population of the nematode. Daincha, green gram, groundnut, sesamum and cowpea proved to be poor hosts of *H.oryzae* (Hebsy *et al.*, 1997).

#### Host Plant Resistance

Ten common varieties were screened under natural conditions at the State Seed Farm for their comparative resistance to infection by sheath blight disease and infestation by the rice root nematode. None of the rice varieties exhibited either resistance to sheath blight or to the nematode. Comparatively, low intensity of disease and nematode infestation was noted in varieties like Bharati, Sabari and Rohini (Gokulapalan, 1981).

#### *Heterodera oryzicola*

The cyst nematode *H. oryzicola* is a serious root infesting nematode of rice in India. It has a very limited distribution being mostly confined to Kerala. The infested plants exhibit root browning and leaf chlorosis and such fields yield poorly. The nematode along with seedling blight fungus *Sclerotium rolfsii* interacts to form a destructive disease complex leading to increased seedling mortality.

#### Distribution

The nematode was first reported from Kerala in 1972. Consequent to the detection

of the noxious pest in the State, survey was undertaken throughout the rice tracts to map its distribution. Initial reports revealed that the nematode was confined to certain districts of the State, the average percentage frequency distribution ranging from 5 to 35. Later, the pest was recorded from all the districts - Thiruvananthapuram (17 per cent), Kollam (14 per cent), Alappuzha (5 per cent), Pathanamthitta (14 per cent), Idukki (40 per cent), Kottayam (5 per cent), Ernakulam (5 per cent), Thrissur (14 per cent), Palakkad (70 per cent), Malappuram (34 per cent), Kozhikkode (6 per cent) Wayanad (71 per cent), Kannur (70 per cent) and Kasaragode (59 per cent). (KAU, 1997). Recently the cyst nematode was seen to be a serious pest of rice in Kannur, Wynad, Palakkad and Idukki districts. Pullari in Kannur, Karimbil and Kodenchery in Wynad, Alathur, Pattambi and Palamcode in Palakkad and Puttadi and Anakkara in Idukki districts were identified as hot spots of the pest (KAU, 2003).

#### Crop Loss

Field level studies revealed an yield loss of 17-42 per cent due to rice cyst nematode (Kumari and Kuriyan, 1981). An initial population level of 210 to 426 larvae per 200g of soil sample showed adverse effect on the biometric characters and yield of the plant. Production of tillers and yield of paddy were reduced by 18 per cent and 19-43 per cent, respectively. In the subsequent year, reduction of 15 to 17 per cent in plant height and 15-16 per cent in productive tillers and subsequently, a loss of 20 to 26 per cent and 12 to 23 per cent in grain and straw, respectively were observed at the same inoculum level.



## Management

The cyst nematode, is a highly destructive pest requiring effective management strategy. Though chemicals were relied on initially for the control of the nematode, the recent research efforts have been concentrated on identifying non- chemical methods. Attempts were also made to identify sources of resistance to the nematode as it is an essential component of integrated nematode management approach.

## Chemicals

Efficacy of different granular nematicides for the management control of rice cyst nematode was tested in the field. In the initial screening trial aldicarb, carbofuran, diazinon, phorate and ethoprop @ 1 kg ai/ha were used. Application of carbofuran, phorate, aldicarb at 7 and 50 days after planting significantly reduced the nematode population which in turn increased the yield of paddy by 29 to 53 per cent. In another trial, application of carbofuran @ 1 kg ai/ha at 7 and 50 D A P effectively reduced the nematode population by 70 per cent and increased the yield by 28 per cent (Jiji *et al.*, 1994). Similarly, application of phorate and carbofuran @ 1 kg ai/ha at 7 and 50 DAP reduced the nematode population and increased the yield of paddy.

## Host plant resistance

Hundred and one varieties/ lines were screened against cyst nematode under glass house conditions. Results revealed the tolerance of two varieties namely TET 7617 and TET 5961. All other varieties were susceptible/highly susceptible.

## *Meloidogyne graminicola*

The nematode is a sedentary endoparasite. The infective juveniles cause disruption

and hypertrophy of cortical cells by intracellular migration and secretion. Depletion in plant vigour, yellowing and curling of leaves along midribs, delay in flowering of crops etc. are the typical foliar symptoms. The nematode causes development of galls throughout the root system. Infested root tips become swollen and hooked which is the characteristic symptom caused by the nematode. While NP and Fe are reduced in infested plants, total sugars and, amine nitrogen are increased (Israel *et al.*, 1963).

## Distribution

The nematode is a well established pest in rainfed upland and sandy, loamy, laterite or alluvial soils. It has been recorded from Orissa, Assam, West Bengal, Madhya Pradesh and Tripura in India and the yield loss has been estimated to be between 16.32 and 64 per cent.

Recently, the nematode was recorded from the rice tracts of Kerala. It has been reported from Kannur, Wynad, Palakkad and Idukki districts, the frequency of occurrence being 0-90 per cent. Heavy infestation was observed in Idukki district where attack in the nursery resulted in complete damage of the crop (KAU 2001). Perumpadavam in Kannur, Kodenchery and Tharuvana in Wynad, Nenmeni, Alathur, Palamkode and Pattambi in Palakkad, Puttadi, Anakkara and Chettukuzhi in Idukki districts have been identified as hot spots of the nematode in the State.

## *Aphelenchoides besseyi*

This nematode is present in rice tracts in the states of Tamil Nadu, Andhra Pradesh, Haryana, Himachal Pradesh and Tripura.

It spreads through seeds and exchange of rice germplasm. During the 1960s, occurrence of the nematode was recorded from Kollam, Palakkad and Kottayam districts in Kerala. The nematode has not been detected from any part of the State, subsequently.

#### Other nematodes

The other nematodes found attacking rice were *Helicotylenchus* spp., *Tylenchorhynchus* sp., *Hoplolaimus* sp. and *Criconemoides* sp., *Caloosia* sp., *Hemicycliophora* sp. and *Aerolaimus* sp.

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# NEMATODES OF VEGETABLES

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Vegetables are an important source of nutritional factors like proteins, vitamins and minerals in human diet and are considered next in importance to cereals or staple crops. With its increase in population, there is ever increasing demand for vegetables and this in turn enable the farmers to get good return for his commodity. Despite the use of high yielding varieties and optimal inputs the production of vegetables is hampered by the attack of plant parasitic nematodes in combination with pests and diseases.

The plant parasitic nematodes of vegetables incur direct and indirect losses to vegetable crops. The first record of nematode damage to vegetables was that of Berkeley (1855) on cucumber by root-knot nematode who described the disease as 'Vibrios forming excrescence'. Almost all the vegetable crops have been found to be susceptible to this nematode. Some important root-knot nematode species reported on vegetables include *M. incognita*, *M. javanica*, *M. arenaria*, *M. aeronea*, *M. africana*, *M. kukugensis*, *M. artiella*, *M. lucknowica*, *M. ethiopica*, *M. exigua*, *M. naasi*, *M. graminicola*, *M. thamesi* and *M. hapla*. *M. hapla* is commonly distributed in cooler areas while *M. incognita* and *M. javanica* in warmer areas. Out of 1000 root-knot populations collected in 75 countries, 53 per cent were identified as *M. incognita*, 30 per cent as *M. javanica*, 8 per cent as *M. arenaria*, 7 per cent as *M. hapla*, 2 per cent as *M. exigua* or other species (Johnson and Fassuliotis, 1984). The cropwise association and its importance are given in Table 1.

Of the 176 nematodes associated with vegetables in India (Mahajan *et al.*, 1986), the major ones are *Meloidogyne* spp., *Rotylenchulus reniformis*, *Heterodera* spp., *Globodera* spp., *Tylenchorhynchus*, *Radopholus similis*, *Tylenchus* spp., *Ditylenchus* spp., *Pseudolenchus* spp., *Hoplolaimus* spp., *Helicotylenchus* spp., *Aphelenchoides* spp., *Seinura* spp. and *Longidorus* spp. (Sakhiya and Jain, 2001). There exists difference between plant parasitic nematode infesting the tropical and temperate vegetables. However, *Meloidogyne* spp. are ubiquitous in distribution. Similarly, *Ditylenchus dipsaci* and species of *Heterodera* which are temperate parasites are also capable of causing damage in localised pockets in warm tropics. Nematodes like *Meloidogyne* spp., *R. similis* and *Heterodera* spp. are widely distributed in Kerala and are found associated with the major vegetable crops grown.

The extent to which nematode damage the vegetable crop depends greatly on the farming system followed. They inflict less damage under extensive and varied growing system such as multiple cropping or under widely spaced crop rotation, whereas, they incur serious crop loss in situations of intensive farming, monocropping and narrow crop rotations. In multiple cropping systems, the nematode population and damage caused is found to build up with advent of time, the extent of the increase being dependant on the initial nematode population and the percentage of susceptible plants per unit are

Table 1. Root-knot nematodes, *Meloidogyne* spp. associated with vegetable crops in subtropics and tropics

Common name	<i>Meloidogyne</i> spp.								
	<i>M.i</i>	<i>M.a</i>	<i>M.j</i>	<i>M.h</i>	<i>M.ac</i>	<i>M.et</i>	<i>M.e</i>	<i>M.t</i>	<i>M.c</i>
Okra (bhindi)	V	V	V						
Onion	V	L	L				U		
Chilli (Sweet pepper)	M	L	U				U	U	
Cucumber	V	V	V	M			L		
Pumpkin	V	V	V						
Squash	V	V	V	M					
Carrot	V	L	V	L					
Egg plant (Brinjal)	V	V	V	L					
Tomato	V	U	V			U	L		U
Bottlegourd	M	M	V						
Spongegourd	V	L	V						
Water melon	V	V	V	M			L		
Melon	V	L	V	M			L		
Cabbage	M	L	L	L					
Cauliflower	L	L	L	L					
Beetroot	L	L	L	L					
Garlic	L	U	L	L					

V - Very important

M - Moderately important

L - Limited or local importance

U - Unknown importance

*M.i* - *M. incognita*

*M.a* - *M. arenaria*

*M.j* - *M. javanica*

*M.h* - *M. hapla*

*M.ac* - *M. acronea*

*M.et* - *M. ethiopica*

*M.e* - *M. exigua*

*M.t* - *M. thamesi*

*M.c* - *M. cruciani*

### Brinjal (*Solanum melongena* L.)

This economically important vegetable crop is severely affected by four species of root-knot nematode, the reniform nematode and three species of ectoparasitic nematodes. Of these, the root-knot nematode causes most concern and incur 26.2 to 50 per cent yield loss. The most characteristic symptom of infestation is the appearance of galls or knots on roots as a result of which the number and size of fruits get reduced and occasionally induce wilting or drooping of mature plants during mid day in peak summer months.

### Crop loss

Yield loss due to root-knot nematode, *M. incognita* in brinjal was estimated by comparing the difference in yield of brinjal fruits (number and weight) in the presence of nematode and nematode free situation by controlling nematodes with the application of carbofuran @ 3 kg a.i ha<sup>-1</sup>. An available yield loss of 20 and 22 per cent in terms of weight of fruits and number of fruits respectively was obtained at an initial nematode population level of 248 *M. incognita* juveniles per 250 g soil. The biometric characters like height of plants and number of leaves

were reduced by 29 and 32 per cent respectively (Sheela *et al.*, 1999)

### Management

Different strategies have been adopted from time to time for the management of the nematode pests of brinjal, the most widely practised being the application of nematicides. Apart from chemicals, solarization of the nursery beds for 15 days with Low Density Polyethylene (LDPE) film having 150 gauge thickness, application of organic amendments and biological control agents like Arbuscular mycorrhizal fungi, *Glomus fasciculatum*, bacterial antagonists like *Bacillus macerans*, *B. circulans*, *B. coagulans*, *Pasteuria penetrans*, *Pseudomonas fluorescens* etc. were also studied for managing nematodes in the nursery to get initial protection to the brinjal seedlings.

### Chemical methods

Though nematicides are generally used for soil application, nursery treatment and soil drenching in the nursery for controlling nematodes, the bare root dip of seedlings of transplanted crops like brinjal, tomato and chilli in nematicidal solution at the time of transplanting emerged as an attractive proposition for avoiding pesticide residue in the agro-ecosystem.

### Nursery bed treatment

To find out the effect of nursery treatment for the control of root-knot nematode in brinjal, the nematicides. Dibromochloropropane (DBCP) @ 3 ml a.i. m<sup>2</sup>, methamsodium (Vapam) @ 15, 20 and 25 ml/m<sup>2</sup> carbofuran (Furadan) @ 0.2, 0.3 and 0.4 g m<sup>2</sup> were tried. Nursery beds were treated with DBCP and metham sodium 15 days before sowing. The other chemicals were applied uniformly on prepared beds and the soil was raked up one day prior to

sowing. Maximum protection from root-knot nematode was obtained in carbofuran treatment @ 0.4 g m<sup>2</sup> followed by aldicarb @ 0.4 g m<sup>2</sup> and metham sodium @ 25 ml m<sup>2</sup>. The chemicals were effective in reducing the nematode population in the roots by 26 to 71 per cent. (Table 2) Carbofuran 0.4 g m<sup>2</sup> was comparatively more effective in controlling the nematode population in root and protecting the seedlings from initial infestation by nematodes which was beneficially reflected on the yield of brinjal (KAU, 1985). Some new chemicals like Phenamiphos 0.3 g m<sup>2</sup>, Dibutylamino sulfanylcarbofuran 0.3 g m<sup>2</sup> and cytolane 0.3 g m<sup>2</sup>. in controlling root-knot nematode in nursery when tested along with the already proven chemicals, carbofuran and metham sodium were found to be equally effective (KAU, 1989). Granular nematicides like quinalphos, phorate, disulfoton @ 0.2 g m<sup>2</sup> were evaluated in brinjal nursery along with carbofuran and aldicarb @ 0.2 g m<sup>2</sup>. These newly tested granular nematicides were inferior to aldicarb in reducing the nematode population and protecting the seedlings from root-knot and increasing the yield. In order to fix the optimum dose of nematicide found effective in initial evaluation trial for the control of root-knot nematode in the brinjal nursery, aldicarb @ 0.2, 0.3 and 0.4 g a.i. m<sup>2</sup>, carbofuran 0.2, 0.3 and 0.4 g a.i. m<sup>2</sup> and UC 54229 @ 0.2 g a.i. m<sup>2</sup> were again tested. Compared to other chemicals and concentrates aldicarb @ 0.3 g a.i. m<sup>2</sup> was most effective in reducing the nematode infestation and increasing the yield (KAU, 1991). Mocap and Carbofuran @ 0.3 and 0.6 g a.i. m<sup>2</sup> were tested in the nursery along with the recommended chemical carbofuran @ 0.3 and 0.6 g a.i. m<sup>2</sup> and revealed that phorate too was effective in managing nematode population in brinjal seedlings and gave better crop yield (KAU, 1993).

Table 2. Effect of nursery treatment on the biometric characters, yield and root-knot formation of brinjal

Treat-ments	Number of leaves		Shoot weight		Root weight		Number of fruits		Weight of fruits		Number of gall	
	Mean (No.)	% increase over check	Mean (Wt.)	% increase over check	Mean (No.)	% increase over check	Mean (Wt.)	% increase over check	Mean (Wt.)	% increase over check	Mean No. of	% decrea over check
T <sub>1</sub>	74.51	11.2	165.45	12.4	40.30	1.2	7.30	8.9	289.05	14.80	579.75	25.9
T <sub>2</sub>	99.90	17.5	168.18	14.8	44.50	9.1	7.58	13.13	01.63	19.80	531.85	31.7
T <sub>3</sub>	120.01	41.2	188.43	28.0	50.64	24.0	8.98	34.0	354.50	40.8	482.30	38.3
T <sub>4</sub>	143.28	68.5	217.58	47.8	55.98	37.4	10.98	63.9	424.80	68.7	332.88	57.4
T <sub>5</sub>	117.70	38.4	184.75	25.5	48.40	18.8	7.98	19.1	309.75	22.9	517.98	33.8
T <sub>6</sub>	133.20	56.7	197.08	33.9	53.55	31.4	9.38	40.0	366.60	45.6	401.70	48.6
T <sub>7</sub>	171.58	101.8	236.05	60.4	58.60	43.8	12.33	84.0	496.65	97.2	225.18	71.2
T <sub>8</sub>	110.65	30.1	174.40	18.5	47.43	16.4	7.95	18.7	302.13	19.9	520.95	33.4
T <sub>9</sub>	122.80	44.4	194.08	31.8	51.40	26.1	9.15	36.6	365.21	45.0	442.90	43.4
T <sub>10</sub>	143.94	69.3	222.45	51.1	57.28	40.4	11.18	66.9	426.98	69.5	247.23	68.4
T <sub>11</sub>	85.03	-	147.20	-	40.75	-	6.7	-	251.85	-	782.00	-
CD at 5%	37.46	-	54.75	-	15.07	-	0.42	-	83.43	-	51.3	-

T<sub>1</sub> DBCP at 3 ml ai/sq.m  
T<sub>2</sub> Metham sodium at 15 ml/sq.m  
T<sub>3</sub> Metham sodium at 20 ml/sq.m  
T<sub>4</sub> Metham sodium at 25 ml/sq.m  
T<sub>5</sub> Carbofuran at 0.2 g/sq.m

T<sub>6</sub> Carbofuran at 0.3 g/sq.m  
T<sub>7</sub> Carbofuran at 0.4 g/sq.m  
T<sub>8</sub> Aldicarb 0.2 g/sq.m  
T<sub>9</sub> Aldicarb 0.3 g/sq.m  
T<sub>10</sub> Aldicarb 0.4 g/sq.m  
T<sub>11</sub> Untreated (check)

### Seedling root dip treatment

Bare root-dip of seedlings of transplanted crops like brinjal is a novel method for affording initial protection of seedlings in integrated nematode management strategy. Root dip of brinjal seedlings in different nematicidal solutions *viz.*, triazophos, carbosulfan, monocrotophos and zolone at 500 and 1000 ppm significantly reduced root infestation by the nematode, the root-knot formation and improved root growth in seedlings. This reduction in infectivity, however, was not reflected in the improvement of

growth characters and yield significantly. Yet, 7 to 43 per cent yield increase could be observed due to various treatments (KAU, 1992). The success obtained in subsequent trials proved the effectiveness of bare root-dip of monocrotophos and carbosulfan @ 500 ppm for the management of root-knot nematode.

### Host resistance

Screening for resistance against root-knot nematode in brinjal revealed that among the 55 varieties/lines evaluated, ten varieties were found tolerant to root-knot while

23 were susceptible, four highly susceptible and 18 moderately resistant. The resistant/tolerant varieties/lines were 363-14-16, Type 3482-2-4-10, 120AAT-7493-9, 480-13-1-2, 480-13-1-2, 449-1-8, 555-8, Ghatika white, Maroo Marvel, SM-6-7 and Pbr-91-2.

### Organic amendments

Organic amendments like oil cakes of castor, mustard and neem and their method of application were evaluated for their efficacy in managing root-knot nematode in brinjal in nematode infested fields. The oil cakes were applied @ 100 g per furrow and 15 g per spot. Nitrogen was added in the form of urea equivalent to the cakes. Cakes were added and allowed to decompose for 15 days. Results showed that the nematode population was reduced 38 to 94 per cent in the amended soil. The highest reduction of nematode population in soil and root-knot count as well as the highest yield was recorded due to incorporation of mustard cake given as spot application followed by neem cake as furrow application. (KAU, 1983).

Spot application of neem cake @ 15 g/spot was comparatively superior to furrow application in controlling root-knot nematode and increasing the yield of brinjal plants. The increase in yield was 98 to 108 per cent in spot application and 7 to 47 per cent in furrow treatment. The nematode population in soil and root-knot production were significantly reduced by the different treatments to a tune of 53 to 82 per cent in soil and 36 to 75 per cent in root (KAU, 1985).

Application of oil cakes of castor, neem and mustard @ 15g per pit and 240g per plot by broad casting and organic wastes like saw

dust, coconut, husk powder, paddy husk, lemon grass waste and cashew shell powder @ 2500kg/ha in the pits or by broad casting significantly increased the yield of brinjal through successful suppression of root-knot nematode population (Kamalakhshamma,1987)

### Trap Crop

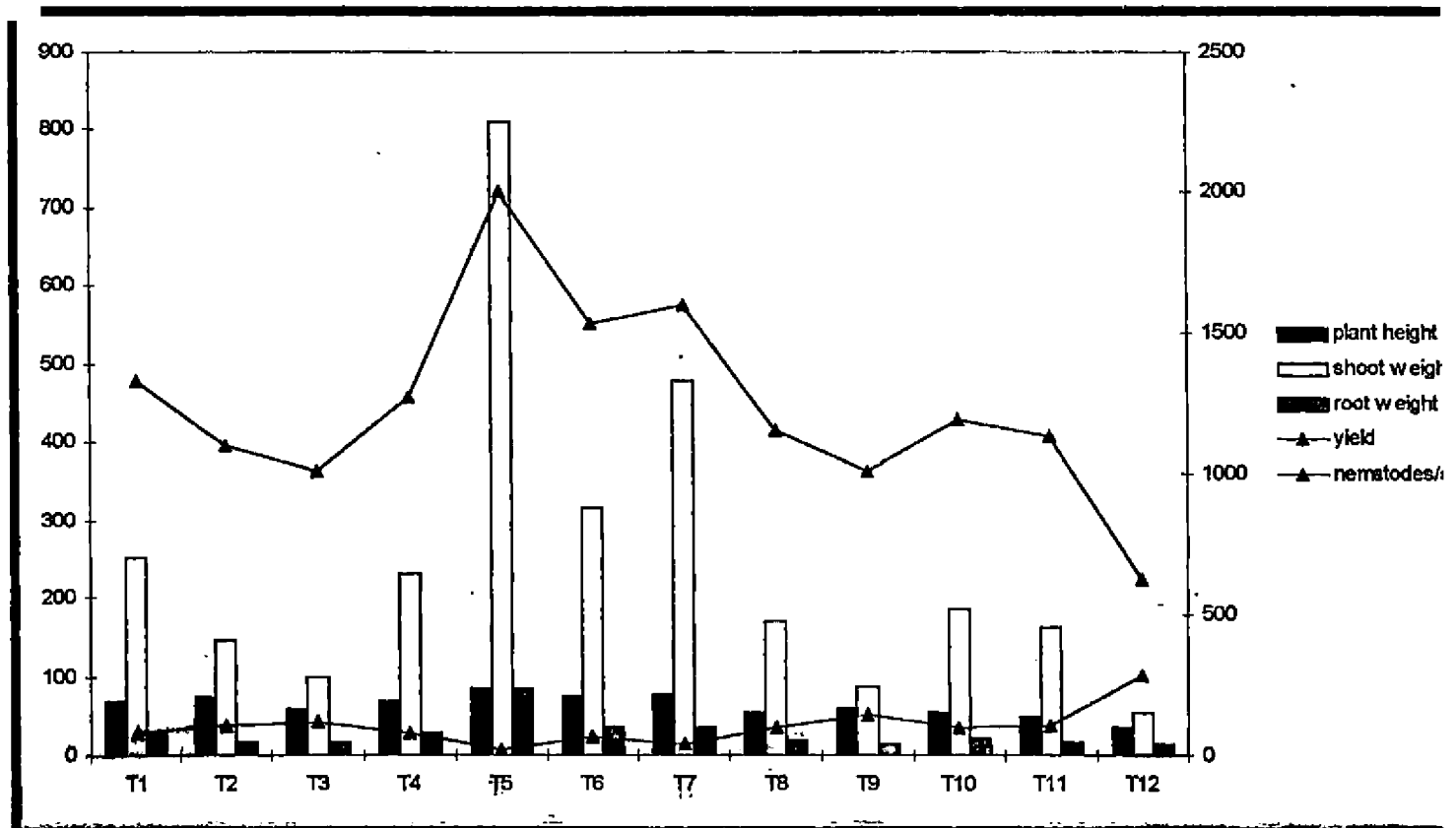
Use of trap crops has been found effective for controlling root-knot nematode in homestead situations. The resistant variety of sweet potato Sree Bhadra reduced the nematode population to a tune of 20-25 per cent in bhindi and brinjal root zone and increased the yield of above crops 20-25 per cent (KAU, 2003). Rotation with this non host crop was also tried in brinjal and bhindi.

### Biological Control

#### *Bacillus macerans/Paecilomyces lilacinus*

Biocontrol agents like *Bacillus macerans* and *Paecilomyces lilacinus* obtained from native soils were found to increase the yield of fruits through suppression of nematodes. Nursery application of these biocontrol agents along with drenching enhanced the fruit weight and performed superior to the recommended chemical application and neem cake incorporation (Sheela and Nisha, 2004).

A mean yield of 85.56 and 81.39 kg/40m<sup>2</sup> was obtained in farmers' participatory research by following the above method using *B. macerans* and *P. lilacinus*, respectively. Among the two agents *B. macerans* gave the highest incremental income to the farmer. Accordingly, application of *B. macerans* by nursery treatment @25g/m<sup>2</sup>+ drenching 2 per cent solution 7DAS has been found to be viable practice under field condition.(Fig.1)



T1 - *P. fluorescens* nursery treatment + drenching, T2 - *P. fluorescens* drenching, T3 - AMF14  
 T4 - AMF15, T5 - *B. macerans* nursery treatment + drenching, T6 - *B. macerans* drenching  
 T7 - *P. lilacinus* nursery treatment + drenching, T8 - *P. lilacinus* drenching, T9 - Carbofuran  
 T10 - Neem leaf, T11 - Neem cake, T12 - Untreated



### AMF application

Arbuscular mycorrhizal fungi like *G. fasciculatum* and *G. constrictum*, the brinjal plants afforded significant protection to brinjal plants from root-knot infestation in the nursery. The treated plants when transplanted to the main field, showed increased growth and fruit yield (Asha, 1996).

### *Pseudomonas fluorescens*

The commercial formulation of *P. fluorescens*, procured from TNAU, Coimbatore was evaluated for the management of root-knot nematode in brinjal nursery and the effects were compared with that of soil solarization of nursery beds using 150 gauge (LDPE) polythene sheets for 15 days during peak summer period, nursery application of *G. fasciculatum* (5g spore medium) @ 200 spore per g medium and neem cake @ 200 /m<sup>2</sup>. The performance of the seedling in the main field revealed that all the treatments were equally effective. The growth of the seedling showed enhancement due to suppression of the native phytonematodes which in turn boosted the fruit yield by 15 to 20 per cent (Sheela *et al.*, 1999).

Combined application of mycorrhizal fungi with biocontrol bacteria like *B. macerans*, *P. fluorescens* and *P. penetrans* synergistically enhanced the growth of plants by 50 to 60 per cent.

### Integrated nematode management

Integration of chemical, cultural, physical, biological, use of resistant varieties and regulatory methods in a harmonious way is needed for better management or maintenance of the nematode population densities below the economic threshold level. For the suppression of root-knot nematode

in brinjal, an attempt was made to integrate some common and recommended practices and to evolve a better management strategy. This involved combinations of the following practices :

Nursery treatment + cultural operations in main field + spot application of nematicides

Deep ploughing + nematicide application

Nursery treatment + solarization

Application of neem cake + granular nematicides

Treated nursery + solarization + use of neem cake

### Effect of nursery treatment + cultural operations + spot application of nematicides

The effect of cultural operations, nursery treatment and spot application of nematicides on the control of *M. incognita* of brinjal was studied in field level. Nursery beds were treated with metham sodium @ 25ml/m<sup>2</sup>. Digging of main field to a depth of 10 cm was considered as normal ploughing and 20 cm as deep ploughing. The seedlings (treated as well as untreated) were transplanted 30 days after sowing. Spot application of aldicarb was done on the day of planting. Besides, various combinations of normal ploughing (10 cms) deep ploughing (20 cm) treated seedling (methamsodium @ 25 ml per m<sup>2</sup>) untreated seedlings, spot application of aldicarb @ 1 kg ai/ha and no application etc. were tried.

Maximum yield of brinjal was obtained under normal ploughing + metham sodium treated seedling + spot application of aldicarb. This was closely followed by deep ploughing + treated seedling + no aldicarb. The root-knot nematodes population in root

and root-knot count (galls) were reduced by deep ploughing + treated seedling + no aldicarb treatment revealing that aldicarb application alone did not have much effect on the reduction of nematode population in the root.

Plants in untreated nursery planted in deeply ploughed plots treated with aldicarb @ 1 kg ai/ha showed maximum increase in height (32 per cent over control). The increase in yield (number and weight of fruit) due to various treatment combinations ranged from 2 to 23 and 2 to 40 per cent respectively. The number of galls and nematode population in soil (*M. incognita*) also showed drastic reduction due to various treatments, the percentage reduction being 21 to 79 and 29 to 54, respectively (KAU, 1983).

#### **Effect of deep ploughing and nematicides**

In order to find out the effect of ploughing, covering the soil with polythene sheet and nursery soil treatment with carbofuran, individually and in combinations, on the root knot nematode infestation, the following treatment combinations were tried.

Ploughed and exposed + treated seedlings (carbofuran 0.3 g ai/m<sup>2</sup>)

Ploughed and exposed + untreated seedlings

No ploughing or covering + treated seedlings

Ploughed and covered + untreated seedlings

Ploughed and covered + treated seedlings

No ploughing or covering + untreated seedlings

Results revealed that the maximum reduction in soil moisture content was observed when plots were ploughed and

exposed, which also resulted in 66 to 77 per cent reduction in soil nematode population. Treated nursery seedlings irrespective of the main field treatments (ploughing, exposure or covering) had lower root-knot nematode population in soil with a corresponding increase in yield. However treatment combinations with ploughing and exposure + treated seedlings gave significantly higher yield (78 per cent increase in weight of fruit).

#### **Effect of nursery treatment + solarization.**

The effect of integrating soil solarization with nursery treatment for managing the root-knot nematode in brinjal was also studied.

#### **Main treatments**

- a. Ploughed and exposed (minimum for two weeks)
- b. Ploughed and covered with transparent polythene (minimum for two weeks) with its edges properly sealed by putting soil over it.
- c. No ploughing / covering

#### **Sub treatments**

- a. Treated nursery (raised in beds applied with carbofuran @ 0.3 g ai/m<sup>2</sup>)
- b. Untreated nursery

The root-knot nematode problem in brinjal can be effectively tackled at the nursery level. (Table 3). Seedlings raised in carbofuran treated nursery when transplanted to the main field established better and were less prone to nematode infestation as indicated by the gall index recorded (1.1 to 1.65 as against 1.57 to 2.05 in untreated nursery). Integration of nursery treatment with main field treatments like ploughing + exposing and ploughing + covering with polythene sheets for 15 days significantly improved the plant growth and yield to a tune of 32 per cent in both cases (KAU, 1995).

Table 3. Effect of various treatments on the nematode population and yield of brinjal  
(Average of four replications)

Sl. No.	Treatments	Treated nursery				Untreated nursery	
		Initial nematode population 200 ml soil	Gall index at harvest	Yield kg/ plot	Percentage increase in yield	Gall index at harvest	Yield kg/ plot
1	T <sub>1</sub> - Ploughed and exposed	96.0	1.65	0.58	31.8	2.05	0.51
2	T <sub>2</sub> - Ploughed and covered with polythene sheet (15 days)	96.0	1.10	0.58	31.8	1.57	0.48
3	T <sub>3</sub> - No ploughing or covering	128.0	1.21	0.44	-	1.66	0.35
	CD	-	0.41	0.12	-	NS	NS

#### Effect of application of neem cake + granular nematicides

The cumulative effect of neem cake and granular systemic nematicides in controlling root-knot nematode in brinjal was studied under field condition. The following set of treatment combinations were tried.

- Aldicarb 1 kg ai/ha + inorganic nitrogen 20 kg per ha (Urea 47.6 kg per ha)
- Aldicarb 1 kg ai/ha + neem cake 200 kg/ha + inorganic nitrogen 10 kg (Urea 23.8 kg per ha)
- Aldicarb 1 kg ai/ha + neem cake 400 kg/ha
- Carbofuran 1 kg ai/ha + inorganic nitrogen 20 kg per ha (Urea 47.6 kg per ha).
- Carbofuran 1 kg/ai ha + neemcake 200 kg/ha + inorganic nitrogen 10 kg/ha (urea 23.8 kg/ha)
- Carbofuran 1 kg ai/ha + neemcake 400 kg/ha
- No nematicides + inorganic nitrogen 20 kg/ha (urea 47.6 kg/ha)

- No nematicides + neemcake 200 kg / ha + inorganic nitrogen 10 kg per ha (Urea 23.8 kg / ha)
- No nematicides + neem cake 400 kg per ha
- Control (untreated)

All the biometric characters (number of leaves, height of plant and root weight) were improved by various treatments. The percentage increase over untreated control ranged from 3 to 25; 8 to 80 and 15 to 59, with respect to the height of plants, number of leaves and weight of root, respectively. Highest yield (number and weight of fruit) was obtained in treatment having carbofuran 1 kg a.i. per ha + neem cake 200 kg per ha + inorganic nitrogen 10 kg per ha.

#### Effect of treated nursery + solarization + neem cake

Efficacy of nursery treatment, solarization and neem cake application alone and their combinations were tried for the management of root-knot nematode in brinjal.

Nursery beds were treated with carbofuran @ 0.3 g a.i./m<sup>2</sup>. Solarization was done for 15 days during peak summer period in the main field. The field was ploughed and covered with polythene sheets for two weeks for assessing the effect without solarization. Neem cake was applied @ 200 and 400 kg per ha at the time of planting.

Results showed that solarization for 15 days + nursery treatment with carbofuran @ 0.3 g ai/m<sup>2</sup> + neem cake treatment @ 200 kg per ha was effective in increasing the yield of brinjal. The treatments, solarization for 15 days and application of carbofuran in the nursery were found to reduce nematode population resulting in increased yield.

### Bhindi (Okra)

This vegetable crop is attacked by two important genera of nematodes viz., the root-knot nematode, *Meloidogyne* spp. and the reniform nematode, *R. reniformis*.

### Crop loss

Sheela *et al.* (1994) found that the root-knot nematode, *M. incognita* significantly affected plant height of bhindi plants to a tune of 32 to 42 per cent reduction at various initial inoculum levels of 100 to 1000 juveniles per 200 g soil sample. This reduction in plant height and the biometric characters accounted for loss in yield of fruits by 9 to 29 per cent under field conditions. Though infestation of reniform nematode, *R. reniformis* was constantly recorded on the crop, the potential yield loss due to this nematode alone was only less than five per cent.

### Management

#### Chemical methods

Several methods have been evolved for the management of these nematodes.

Eventhough chemical method causes environmental concerns, it is the popular and traditional method of control of nematode and is recommended in certain occasions where other methods are not feasible. Chemicals can be applied either as seed treatment or pit application or broadcast uniformly in plots. Seed treatment is the cheapest and most effective and economic method for managing nematodes infesting bhindi (okra). Pre- and post- emergence damping off disease was also associated with bhindi crop in the early stage. For the management of both these problems, nematicides like carbofuran and aldoxycarb were applied @ 3 per cent w/w of bhindi seeds, alone and in combination with fungicides like captofol, carbendazim and thiram @ 0.2 per cent (Table 4). There was improvement in growth parameters of plants (height, shoot and root weight) but statistically significant improvement was observed in plant height in aldoxycarb and its combinations with above three fungicides. There was drastic reduction in post emergence damping off at 14 days after sowing. Minimum incidence of the disease was observed in aldoxycarb + carbendazim treatment. Reduction in nematode population was maximum in carbofuran + thiram treatment (89 per cent) followed by aldoxycarb alone with 86 per cent reduction. Maximum yield of bhindi was obtained in seed treatment with aldoxycarb + captofol followed by carbofuran alone which resulted in yield increase of 65 and 62 per cent, respectively (KAU, 1982). In another seed treatment trial with nematicides and fungicides, carbofuran 3 per cent w/w + thiram 0.2 per cent w/w treatment produced highest yield (55 per cent increase) and maximum reduction (91 per cent) in population of nematode in the roots (KAU, 1985).

Table 4. Effect of seed treatment with nematicides and fungicides on the yield and nematode population in bhindi root zone

Treatments	No. of fruits per plot	Percentage increase over untreated	Weight of fruits (kg)	Percentage increase over untreated	Number of galls (5 g root)	Percentage reduction over untreated	Nematode population in soil (200g)	Percentage reduction
T <sub>1</sub>	109.69	25.60	2.166	33.79	25.93	74.24	34.67	75.12
T <sub>2</sub>	111.67	27.89	2.177	34.47	19.20	80.92	35.67	74.39
T <sub>3</sub>	118.33	35.49	2.245	38.67	29.13	71.06	29.33	78.95
T <sub>4</sub>	116.33	33.21	2.331	43.89	21.13	79.01	31.67	77.27
T <sub>5</sub>	120.00	37.41	2.767	70.91	23.71	76.43	28.00	79.95
T <sub>6</sub>	108.33	24.05	2.139	32.12	34.87	65.36	46.33	66.75
T <sub>7</sub>	135.00	54.59	3.306	104.20	9.00	91.06	29.67	78.71
T <sub>8</sub>	105.00	20.23	2.029	25.32	15.93	84.18	33.00	76.32
T <sub>9</sub>	87.33	-	1.619	-	100.67	-	139.33	-
CD	21.98	-	0.487	-	19.18	-	1.389	-

#### Treatments

T<sub>1</sub> – Aldicarb sulfone 3 % a.i. (w/w)

T<sub>2</sub> – Carbofuran 3 % a.i. (w/w)

T<sub>3</sub> – Thiram 0.2 % a.i. (w/w)

T<sub>4</sub> – Carbendazim 0.2 % a.i. (w/w)

T<sub>5</sub> – Aldicarb sulfone 3 % a.i. (w/w) plus Thiram 0.2 % a.i. (w/w)

T<sub>6</sub> – Aldicarb sulfone 3 % a.i. (w/w) plus Carbendazim 0.2 % a.i. (w/w)

T<sub>7</sub> – Carbofuran 3 % a.i. (w/w) plus Thiram 0.2 % a.i. (w/w)

T<sub>8</sub> – Carbofuran 3 % a.i. (w/w) plus Carbendazim 0.2 % a.i. (w/w)

T<sub>9</sub> – Untreated (check)

Seed soaking techniques were also tried to control nematodes associated with bhindi. Seeds were soaked in 0.1 per cent solutions of oncol, marhal, dimethoate, dimecron and monocrotophos for six hours. All the above chemicals numerically increased the yield to the tune of 4-16 per cent but was not significantly more than the untreated control (KAU, 1989).

Seed dressing and seed soaking techniques studied in detail during 1991 revealed that carbosulfan (25.ST) seed dressing @ 3 per cent w/w of seed for 90 min was

very effective in improving the biometric characters and yield of bhindi (20-35 per cent). For confirming the results, demonstration trials were conducted in the farmers field at different locations in summer ploughed fields. The results revealed that there was three fold increase in yield of brinjal at the various locations due to this treatment alone (KAU, 2005).

In order to avoid the application of chemicals in vegetables, research efforts are also directed towards the application of green leaves like neem and eupatorium having

nematicidal properties. Application of green leaves of neem and eupatorium @ 300 g per pit reduced the *M. incognita* population below 50 per cent in the root zone of bhindi during summer and rainy season (Ajith, 1992). The predatory and saprophytic nematodes in the root zone increased significantly due to incorporation of leaves 15 days before sowing which in turn suppressed the plant parasitic fauna (Ajith *et al.*, 1993). The effect of this application persisted for 75 days after application. Pre-sowing application of eupatorium leaf significantly boosted the build up of both fungal and actinomycetes population in the rhizosphere. Neem leaf application also had a suppressing effect (Ajith and Sheela, 1996).

### Host resistance

Host resistance is an essential component of integrated nematode management approach. Screening of available germplasm of bhindi against root-knot nematode yielded valuable information on the sources of resistance and the following varieties showed resistance/tolerance under field conditions.

IC 9825, IC-9857, IC 9858, IC 169404, IC 22250, IC 27868, IC 329364, EC 169357, EC 16939, EC 169334, EC 169357, EC 329369, EC 329371 and IARB 02 (KAU, 1999). K-117306, K-198370, K-19837, K-117297 (2001). NBPGR-TCR 770, NBPGR-TCR-852, NBPGR-TCR-937 (KAU, 2003).

### Crop rotation

Crop rotation studies revealed that when bhindi and brinjal raised in heavily root-knot infested field after sweet potato var. Sreebhadra, the population of nematodes in soil was reduced to a tune of 21 and

47 per cent, respectively and this reduction in initial population of nematode was reflected in the increase in yield of bhindi and brinjal crop to a tune of 21-28 per cent (KAU, 2001).

### Biological control

A comparative field trial with AMF, *B.macerans*, *P.fluorescens* and *P.lilacinus* by seed dressing @ 3% w/w and drenching @ 2% revealed that the bioagents effectively reduced the population of nematodes by 90 – 95% in root and 93 – 99% in soil which statistically on par with chemical application of carbofuran @ 1 kg ai / ha. *B.macerans* seed dressing and drenching 7 DAS caused significant yield increase over the chemical treatment. The benefit-cost ratio was also high in *B.macerans* and *P.lilacinus* treatment with carbofuran and neem cake application.

Combined application of AMF with bacteria like *B.macerans*, *P.fluorescens* and *P. peretans* exerted a synergistic effect on improving the biometric character and yield of bhindi crop to the extent of 30 – 35 per cent.

### Chilli

About 30 species of plant parasitic nematodes are associated with *Capsicum* sp. (Sitaramaiah, 1984), among which three species of *Meloidogyne* i.e., *M. incognita*, *M. arenaria* and *M. graminicola* (Rao *et al.*, 1984) and the spiral nematode *Helicotylenchus dihystra*, the stunt nematode, *Tylenchorhynchus* sp. and root-lesion nematode, *Pratylenchus* sp. are the most important.

Twenty two varieties/lines of chilli were screened against root-knot nematode. Among the twenty two varieties/lines evaluated nine were tolerant to root-knot nematode while rest were susceptible. The tolerant varieties were C-70A, G-96-4-9-3-1, G-82-2-1-3, CA-1068, CA-2123, B-70-A, CA-2057, CA-2104 and CA-2210 (KAU, 1997).

## Tomato (*Lycopersicon esculentum*)

It is known for its outstanding nutritive value, is one of the most popular and widely grown vegetables in the world. Several nematodes are associated with the crop of which the root-knot nematode, *Meloidogyne* spp and reniform nematode, *R. reniformis* are the important pests. Root-knot nematode caused 28.0 to 47.5 per cent yield loss in tomato. Considering its polyphagous nature and the economic importance of the nematode, research works have been taken in India and abroad for minimizing the damage incurred by these nematodes.

## Management

### Organic amendments

Organic amendments selectively foster the multiplication of antagonistic nematodes particularly predaceous forms in the environment and are therefore considered to be an adjunct to biological control of phytoparasitic nematodes in vegetables. Such amendments often augment the native saprophytic biocontrol fungi and bacteria. Galling of tomato roots by *M.incognita* was reduced by 75-99 per cent on plants raised in soil mulched with flax, lucerne or orchid grass residue (Joseph, 1972).

### Host resistance

Most of the tomato varieties / lines cultivated in Kerala were badly affected by root-knot nematode, *M. incognita*. Screening for resistance to this nematode revealed that among the thirty eight varieties/lines evaluated twenty were tolerant to root-knot nematode while the rest were susceptible/highly susceptible. The tolerant varieties are Rossol, VFN-8, Phenni (Punnui 442), Campbell-25, Sonita, SL-120, F-38-E2, Kewalo, Anahu, Marsol, Martarum, Punjab NR-7, Montabo, Ronita, Ace, PAU-1 (7-3-1-4), PAU-2

(1-6-1-4), PAU-3 (3-6-3-1-7), PAU-4 (8-2-1-2-5) and PAU-5 (2-1-2-4) (KAU, 1997).

### Integrated management

Integrated management of root-knot nematodes by nursery solarization and botanical materials like green leaves of *Ipomea*, congress grass, eupatorium, neem leaf and calotropis revealed drastic improvement in biometric characters at the seedling stage and enhanced growth and yield of tomato fruits in the mature plant. There was three fold increase in yield of fruits in plots receiving solarization and neem leaf incorporation (KAU, 2005)

### Cowpea/Pulses

Some of the important plant parasitic nematodes recorded on this crop are *Meloidogyne* spp., *Heterodera* spp. *Pratylenchus* spp. and *R. reniformis*. In Kerala, the root-knot nematode (*M.incognita*) and the reniform nematode (*R. reniformis*) have been recognized as the major endemic nematode pest of cowpea.

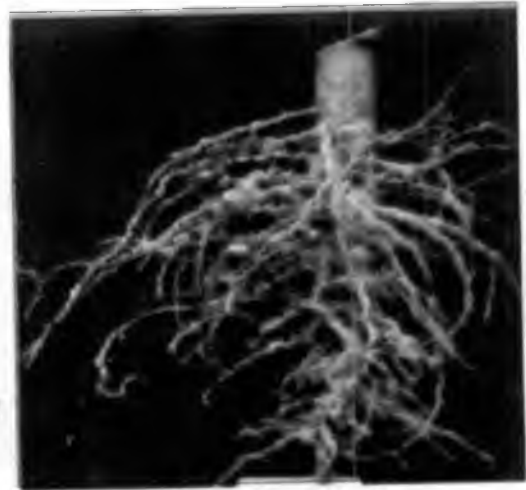
### Crop loss

Inoculum levels of 100,200,400 and 600 larvae / 100 ml soil of *R. reniformis* caused significant debilitating effect. An inoculum level of 400 second stage larvae of *M.incognita*, followed by inoculation of 400 larvae of *R. reniformis* 15 days later, exerted a significant decrease in growth and yield of cowpea (Anitha, 1989).

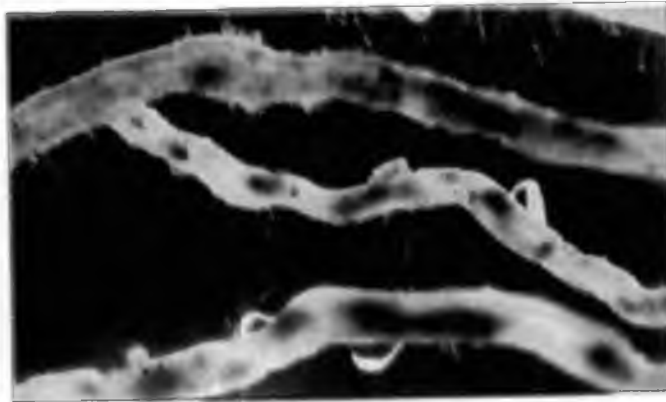
Avoidable loss in yield due nematode infestation in cowpea was assessed by comparing the yield in infested field and treated field (carbofuran @ 2 kg ai ha<sup>-1</sup>). An initial field population of *M.incognita* (270 larvae per 200g of soil sample) along with *R.reniformis* (273 / 200g) reduced the yield of pods by 33 per cent. When population



Root-knots in tomato



Root-knots in bhindi



*R. reniformis* in banana



*Bacillus macerans*



*Paecilomyces lilacinus*

Microbial formulations



ranged from 117 to 413 the reduction in pod yield was 7.7 to 40 per cent. An initial *R.reniformis* population of 78 to 196 per 200 g resulted in an yield loss of 7 to 37 per cent.

The combined infestation of *M.incognita* (211 per 200 g) and *R.reniformis* (178 per 200 g) at the initial stage of the crop reduced the grain yield of cowpea up to 41 per cent (KAU, 2001)

## Management

### Chemical methods

Application of carbofuran 2kg ai/ha at sowing of cowpea seeds suppressed *M.incognita* and *R.reniformis* and improved the biometric characters and yield in terms of pod weight by 20 per cent (KAU, 2000). Seed treatment/seed soaking with nimbicidine, carbosulfan, neem seed kernal and monocrotophos @ 0.1 per cent also effectively managed the nematode population in root in the early stage of the crop which was reflected in enhanced growth and yield (KAU, 2001).

### Biological control

The arbuscular mycorrhizal fungi (AMF), *G.fasciculatum* significantly reduced juvenile penetration and development of the reniform nematodes *R.reniformis* in cowpea. The development of gelatinous matrix was delayed and fewer eggs per egg mass were produced on such plants indicating suppression of fecundity (Deepthy, 1993).

### Host resistance

Mungbean varieties/ lines such as ML3, ML 62, ML 68, ML 70, ML 80, PIMS-1, PIMS-2, PIMS-3 and PIMS-4 were moderately resistant to *M.incognita*. (KAU, 1997).

### Amaranthus

The root-knot nematode, *M. incognita*

and other species are the most important nematode pests of amaranthus. The infestation results in under developed plants, reduction in leaf size and mid day wilting

The role of mycorrhizal fungi in the management of nematodes and biomass enhancement has been authenticated in amaranthus. Cultures of *G.fasciculatum*, *G.etunicatum*, *G.constrictum*, *G.mossae*, *G.monosporum* and *Acaulospora morroweae* significantly reduced the number of galls. The nematode population in soil and root system was significantly reduced in the AMF inoculated nurseries. *G.fasciculatum* was superior in ensuring protection to the plants from the infestation by the root – knot nematode (Asha *et al.*, 1998)

### Gourds

Gourds like bittergourd, ashgourd, bottle gourd are attacked mainly by the root-knot nematodes, *M. incognita*, *M. javanica* and the reniform nematode *R. reniformis* (Hussaini and Saxena, 1969; Sethi *et al.*, 1964; Siddiqui *et al.*, 1974). The root-knot infestation results in poor growth of vines, reduction in size of leaves and crinkling of leaves.

Screening of varieties/ lines of cucumber varieties revealed that ARC-1 was resistant to *M.incognita* (KAU, 1997). The pumpkin varieties Arka Chandran, Hyb-1, S-124 and CM-14 were resistant to *M.incognita* (KAU, 1997).

### Amorphophallus

This crop is prone to attack by a number of nematode pests, the predominantly occurring species being *M.incognita* and *Scutellonema bradys*. These nematodes can be effectively managed by treating the seed material with talc based formulation of *B.macerans* @ 3 g per kg of seed material,

which intum, improved the yield two to three fold. (Sheela *et al.*, 2005).

### Mushrooms

Nematode reduce the market value of mushrooms and heavy infestation results in total loss of crop. Nematodes occur both in compost and in casing. *Ditylenchus* was the first nematode reported to infest the mycelium of mushroom (Lampert *et al.*, 1969). The disease was known as Cephalothecum disease. Other nematodes reported are *Aphelenchoides coprophilus*, *Aphelenchus avenae*, *Ditylenchus myceliophagus*, *A. composticola*, *A. limberti* and *A. winchesi*. In addition, the saprophytic form *Rhabdittis* is reported to carry the bacterium *Pseudomonas tolaasii*, that causes bacterial blotch in mushrooms.

### Conclusion

A large number of plant parasitic nematodes have been recorded from the rhizosphere and roots of vegetable crops some of which inflict serious damage and pose threat to vegetable crops.. The most common and serious nematode pest in these crops appear to be the root-knot nematode, *Meloidogyne* spp. An appraisal of the work conducted reveal that reasonably good progress has been attained in the diagnosis of nematode problems and in evaluating the methods for the management. Most of the research has been centered around the root knot nematode. The pathogenic effects, crop loss (damage) and management through chemical, biological, cultural and integrated methods have been carried out in detail with respect to this nematode. Similar well-laid out experiments should be undertaken to unveil the pathogenic potential and management of other plant parasitic nematodes, especially the reniform nematode. Concerted efforts on the screening and development of

resistant varieties against nematode pests in the major vegetable crops are required. Emphasis should also be laid on the development of Integrated Nematode Management (INM) strategies encompassing the various proven methods. With greater reliance on organic farming these days, efforts for harnessing the potential of biocontrol agents and for development of improved strains and effective formulations for field application should be intensified.

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# NEMATODES OF COCONUT AND ARECANUT

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## Coconut

Many different nematodes have been found in diverse forms of association with the living coconut palm, others have been found associated in different types of symbiosis with insect visitors of the palm, operating and existing in various niches (Govindankutty & Koshy 1979; Koshy & Banu 2002) but the major nematode disease affecting the crop is red ring disease caused by *Bursaphelenchus cocophilus*. The only other nematode known to cause severe damage leading to malfunction in the coconut is *Radopholus similis* (Koshy *et al.*, 1975).

### *Radopholus similis*

The burrowing nematode, *R. similis*, occurs in most tropical and subtropical areas of the world and has been reported from coconut palms in Florida, Jamaica, Sri Lanka and India (Van Weerd *et al.*, 1959a, 1959b; Ekanayake, 1964; Latta, 1966; Weischer, 1967; Koshy *et al.*, 1975). Koshy (1986b) suggested co-evolution of the nematodes along with black pepper and certain cultivars of banana in the western hills of South India. It occurs deep inside the forests on wild black pepper and is widespread on a number of crops like coconut, arecanut, black pepper, banana, betel vine, ginger, etc., in South India.

### Symptoms of damage

The burrowing nematode causes

non-specific general decline symptoms such as stunting, yellowing, reduction in number and size of leaves and leaflets, delay in flowering, button shedding and reduced yield. *R. similis* infestation produces small, elongate, orange-coloured lesions on tender creamy-white roots. Consequent to nematode parasitisation and multiplication, these lesions enlarge and coalesce to cause extensive rotting of the roots. Tender roots of coconut seedlings with heavy infestation become spongy in texture. Surface cracks develop on the semi-hard orange-coloured main roots. Lesions and rotting are confined to the tender portions of the root. Lesions are also not conspicuous on the secondary and tertiary roots since these are narrow and rot quickly on infestation.

As many as 4000 nematodes are known to occur in one g (2.5 cm length) of main roots. The nematode also attacks the plumule, leaf bases and haustoria of seedlings. The above-ground symptoms being non-specific, the only definite method to identify an infested palm is to look for characteristic lesions on fresh, creamy-white to orange-coloured tender main roots after cleaning and rubbing the epidermis.

*R. similis* does not enter or penetrate the coconut roots that have developed a hardened or suberised epidermis but does penetrate the absorbing region behind the root-cap covered by very delicate epidermis

by lysis of cells. The cavities that form in the outer cortex are always surrounded by deeply stained and heavily suberised cells of irregular shape, whereas those formed in the inner cortex do not have any such deformed darkly stained border cells. Maximum number of nematodes and cavities are seen in the outer cortex. Nematodes have not been observed in the stelar region or in the closely packed four to six layers of cells outside the endodermis even in heavily infested roots. In the early stage of infection, roots have separate cavities that later merge with each other consequent to feeding and multiplication of nematodes.

Multiple cavities and their coalescence destroy the cortex to a great extent, but the stelar tube remains intact. Eggs and all stages of nematodes with different orientations are seen in the cavities in longitudinal sections (Koshy & Sosamma, 1982a, 1987; Sosamma & Koshy 1991, 1998).

### **Biology and life cycle**

The burrowing nematode is a migratory endoparasite and is capable of spending its entire life within roots. Most juvenile stages and adult females including gravid females infest healthy succulent root tips; fourth stage and adult males do not. The nematode takes 25 days at 25°- 28°C to complete one life cycle (J2 to J2) (Geetha, 1991).

The coconut isolate of *R. similis* from Kerala, India is the "banana race" as they do not infest *Citrus* spp. or *Poncirus trifoliata* (Koshy & Sosamma, 1977) and has a haploid number four ( $n=4$ ) of chromosomes (Koshy, 1986b; Jasy, 1991). The *R. similis* population from coconut root is easily cultured axenically on carrot discs placed on one per cent water agar or 10% tapioca pearl (Koshy & Sosamma, 1980; Banu &

Sosamma, 1998). It can also be cultured within the mesocarp of growing tender coconuts without affecting the size or quality of the nuts (Koshy & Sosamma, 1982b).

### **Survival and means of dissemination**

The burrowing nematode survives under field conditions for six months in moist soil (27 to 36°C) and one month in dry soil (29 to 39°C); it survives for 15 months in moist soil (25.5 to 28.5°C) and three months in dry soil (27 to 31°C) under glasshouse conditions. The nematode survives in roots of stumps of felled coconut palms for up to six months (Sosamma & Koshy, 1986) and as adult females in coconut roots and soil during summer months causing annual recurrence of infection (Sosamma, 1984.).

Coconut seedlings are raised by sowing seed nuts in the interspaces in coconut plantations in Kerala, India. Most of the nurseries in Kerala and Tamil Nadu (South India) are infested by *R. similis* (Sundararaju *et al.*, 1995). One-year-old coconut saplings raised in these infested nurseries harbour large populations of the nematode in roots internal and external to the husk. Such seedlings when distributed for planting help in the dissemination of the nematode over long distances (Koshy & Sosamma, 1979).

### **Environmental factors affecting parasitism**

Infested coconut roots yield a maximum number of *R. similis* during October to November and minimum during March to July in India. Factors favourable to nematode multiplication are a mean soil temperature below 25°C and a light rainfall coupled with availability of tender fleshy roots. Nematode populations in roots of individual palms were found to vary considerably during low and high peaks depending upon the age, cultivar and conditions of the palms

involved (Koshy & Sosamma, 1978a). The burrowing nematode multiplies well on coconut in loamy sand followed by riverine alluvium, but least in Kari type soils. However, it causes maximum plant damage in riverine alluvium and the lowest in laterite soil (Sosamma, 1984; Sosamma & Koshy, 1985).

#### Other hosts

The coconut isolate of *R. similis* has a wide host range including several economically important plants, weeds and trees. Of 115 plant species tested, 48 species belonging to 45 genera in seventeen families were recorded as hosts (Koshy & Sosamma, 1975; Sosamma & Koshy, 1977, 1981).

#### Disease complexes

The fungi *Cylindrocarpon effusum*, *C. lucidum* and *Cylindrocladium clavatum* have been recorded in association with lesions produced by *R. similis* in coconut roots. In pathogenicity studies, the fungus *C. effusum* did not cause any appreciable damage to inoculated seedlings. The fungus, when inoculated simultaneously with the nematode, reduced the rate of multiplication of the nematode and damage to coconut seedlings (Sosamma & Koshy, 1978, 1983; Koshy & Sosamma, 1987; Sosamma, 2000 b). *Aphelenchoides aligarhiensis*, *Panagrolaimus rigidus* and *Rhabditis* sp. were isolated from leaf rot disease affected spindle leaves of coconut in Kerala, India (Nadakkal, 1965; Sosamma 2000c). Application of Phorate @2g a.i./palm to the base of the unopened spear leaf is found helpful in control of the disease. However the role of nematodes in the disease complex as a passive vector / synergist is yet to be defined (Koshy 2000a; Koshy *et al.*, 2002).

#### Economic importance and population damage threshold levels

Surveys of different coconut growing tracts of Kerala, Karnataka and Tamil Nadu States of India (964 000 ha) revealed the widespread occurrence of *R. similis*. Twenty-four per cent of the root samples yielded *R. similis*, and, of these, 50% yielded one or more *R. similis*/g of root (Koshy *et al.*, 1978; Sosamma, 1984). Thirty per cent increase in yield was recorded by application of *Hydnocarpus* sp. oil cake at 8 kg/palm/year or phorate and aldicarb at 10 g a.i./palm in June-July and October-November to the burrowing nematode infested coconut palms (Koshy, 1986b).

The pathogenicity of *R. similis* on coconut was established by conducting two experiments, the first with duration of five years and the second over a period of one year. An initial inoculum level of 62,500 nematodes per seedling caused 4, 22, 76, 18, 25, 40, 48, and 79 per cent reduction with respect to height, girth at collar region, shoot weight, number of leaves, number of leaflets/leaf, leaflet length, lamina length and root weight over control plants. Effect of parasitization of the nematode was more pronounced on the root system, especially on the number and mass of feeder roots. The threshold inoculum density required for causing significant reduction of various growth parameters was 100 nematodes in 625 cm<sup>3</sup> or 900 g soil under field conditions over a period of five years.

In the second experiment an initial inoculum level of 100,000 nematodes caused 40, 55, 20, 65, 20, 48, and 52 per cent reduction with respect to height, shoot weight, number of leaves, leaf area, number of lateral roots, volume and weight of roots over control plants over a period of one year. Leaf

bases and haustoria of seedlings were also infested by nematodes. No appreciable damage was noticed in plants inoculated with the fungus, *Cylindrocarpon effusum* alone. The pathogenic threshold level of Axenic *R. similis* population for causing damage to all plant growth parameters was 1000 nematodes per seedling or 10 nematodes per 100 cm<sup>3</sup> or 140 g of sandy loam soil under greenhouse conditions. Histopathology of infested roots recorded the presence of nematodes in the cortex in the inter and intracellular positions (Koshy & Sosamma 1983; Sosamma, 1984; Koshy & Sosamma, 1987).

To facilitate normal growth of the plant to flower and exhibit the disease under natural conditions, a detailed pathogenicity trial was initiated in 1.8 m x 1.8 m x 1.2 m field tanks (microplots) over a period of 11 years using axenic inoculum. This experiment, the first of its kind on a perennial crop, clearly brought out the damage potential of burrowing nematode on coconut. All the uninoculated palms came to flowering during 65 to 83 months after planting, between the 31<sup>st</sup> to 49<sup>th</sup> leaf axils whereas four out of the five palms that received an initial inoculum level of 100 nematodes flowered during 67 to 130 months in the leaf axils from 39 to 56. Two palms each that received an initial inoculum level of 1000 and 10,000 nematodes came to flowering after 108 months and one out of five palms that received an initial inoculum level of 1 lakh (100,000) nematodes also came to flowering after 132 months. None of the palms that received 10 lakh (1,000,000) nematodes came to flowering. The control palms produced a total of 155 inflorescences compared to 67 inflorescences in palms inoculated with 100 nematodes as an initial inoculum level. However, the palms that received an initial inoculum of 1000 nematodes and above did

not yield any nuts even eleven years after planting. The control plants produced an average of 125 nuts compared to 37 nuts by palms that were inoculated initially with 100 nematodes. Even one nematode in 35,640cm<sup>3</sup> of soil or 100 nematodes per seedling reduced the yield by 77% (Koshy & Sosamma, 1994,1996).

### Control

Control of the burrowing nematode on a perennial palm such as coconut with a massive root system is difficult, especially under the high density multispecies cropping system that exists along the West Coast of South India involving susceptible crops like arecanut, banana, black pepper, betel vine, ginger, turmeric, etc. Unlimited use of nematicides for the control of the burrowing nematode may cause problems of residual toxicity in coconut water and copra (Habebullah *et al.*, 1983). Apart from this, it may also lead to residual toxicity in the products of the intercrops. Therefore, control of nematodes by field application of nematicides alone is not a practical proposition.

### Cultural practices

The cultural practices existing in Kerala and Karnataka (India) are the application of neem/marotti (*Hydrocarpus*) oil cakes at 2-4 kg/palm/year, farmyard manure@50kg/palm/year, and green foliage and tender stem of *Glyricidia maculata* to the basins @50kg/palm/year. The growing of green manure crops like cowpea, *Crotolaria* or *Sesbania* in the basins and interfaces during June to August and ploughing in of the entire crop at flowering help in reducing the burrowing nematode population and enriching the nutritional status of the soil. In addition, the growing of intercrops like cacao that enriched the soil with sizeable quantities of shed



foliage which helps in the build-up of beneficial organisms and antagonistic micro organisms that may inhibit nematode multiplication (Koshy *et al.*, 1991 a & b; Koshy *et al.*, 2002).

### Biological

Significant increase in width and leaf area has been recorded in coconut seedling that received mycorrhizae alone. Increase in shoot weight, root weight and decrease in lesion indices occurs in seedlings inoculated with mycorrhizae prior to *R. similis*. Prior inoculation of mycorrhizae is more effective against *R. similis* than simultaneous inoculation of mycorrhizae and nematode even though root infection and spore production is slightly reduced in nematode inoculated plants, the lesion index and root nematode population can be successfully lowered with mycorrhizae infection. A mixture of mycorrhizae consisting of multiple endophytes, viz. *Acaulospora bireticulata*, *Glomus fasciculatum*, *G. macrocarpum*, *G. mosseae*, *G. versiforme*, *Sclerocystis rubiformis* and *Scutellospora nigra* was found effective in improving the plant growth and reducing *R. similis* infestation of coconut seedlings (Sosamma, 1994)

Minimum growth characters and maximum multiplication of nematodes were recorded in plants that were inoculated with *R. similis* alone. In combined inoculation of mycorrhizae and nematode, maximum growth is recorded in plants inoculated with *A. bireticulata*. The mycorrhizae, *A. bireticulata* have maximum multiplication on coconut compared to *G. macrocarpum*, *Scutellospora coralloidea* and *S. rubiformis* in nematode-free as well as nematode-inoculated plants. Nematode population is also low in plants inoculated with *A. bireticulata* (Sosamma, 1994; Sosamma *et al.*, 1998).

A new isolate of *Pasteuria* parasitising *R. similis* in Kerala, India has great potential for use in IPM. The infective propagules of *Pasteuria* adhered to the cuticle of adult males, females and juveniles of *R. similis* (Sosamma, 1999, 2000b,d, 2002). Introduction of *Paecilomyces lilacinus*, *Pasteuria penetrans* and mycorrhizae into potting mixture contained in plastic bags of coconut nurseries and again in the planting pit at the time of transplantation of coconut seedlings in the field helps in better establishment of plants and imparts better growth by offering protection against *R. similis* (Koshy, 1998; Koshy *et al.*, 1998 a; Sosamma *et al.*, 1998). *Catenaria vermicola* was also found parasitising *R. similis* in Kerala (Sosamma, 2000 a)

Introduction of *Paecilomyces lilacinus*, *Pasteuria penetrans* and Vesicular Arbuscular Mycorrhizae (VAM) in to potting mixture contained in poly bags of coconut nurseries and again in planting pit at the time of transplantation of coconut seedlings in the field helps in better establishment of plants and imparts better growth by offering protection against *R. similis* (Koshy 1998; Sosamma *et al.*, 1998).

### Resistance and tolerance

All the coconut cultivars (29 exotic, 15 indigenous and 15 hybrids) screened for resistance to *R. similis* in India were found susceptible in varying intensities. The dwarf cultivars; Kenthali and Klappawangi, recorded the least nematode multiplication and lesion indices. Similar reactions were noticed in hybrids such as Java Giant x Kulasekharam Dwarf Yellow, Kulasekharam Dwarf Yellow x Java Giant, Java Tall x Malayan Yellow Dwarf and San Ramon x Gangabondam (Sosamma *et al.*, 1980, 1988; Sosamma, 1984).

## Chemical

Burrowing nematode infestation in coconut nurseries has been detected in India. Increased incidence of *R. similis* can occur when banana is used as a shade crop in coconut nurseries. In these situations, there is possibly a case for treatment of nurseries with nematicides to produce nematode free seedlings to prevent spread of the nematode into the main field and to uninfested areas.

Past experience has shown that a dip in 1000 ppm DBCP for fifteen min is effective in controlling nematodes in seedlings for *R. similis* infested coconut nurseries (Koshy & Sosamma, 1979). Complete control of *R. similis* can be obtained with soil application of phenamiphos or phorate at 25 kg a.i./ha during September, December and May in infested coconut nurseries (Koshy & Nair, 1979; Koshy *et al.*, 1985).

## Summary of control measures

The following measures are suggested towards developing an integrated management schedule for *R. similis* infestation on coconut palms (Koshy 2002).

1. Application of cow dung (50kg), oil cakes (2-4kg) and green manuring with *Glyricidia maculata* (50kg) /palm/year to the basins.
2. Growing *Crotalaria juncea*, cowpea or *Sesbania* in the basins and interspaces and incorporating into the soil by ploughing in at flowering stage.
3. Application of phorate at 10 g a.i./palm twice yearly (in June-July and in October-November in India).
4. Avoid growing of bananas as a shade crop in coconut nurseries.
5. Use of nematode free planting material of coconut and other intercrops.

6. Use of tolerant or less susceptible cultivars or their hybrids in infested areas.
7. Cut and remove all roots external to the husk of seedlings raised in the field before planting.
8. Raise coconut seedlings in potting mixture enriched with bioagents such as *P. lilacimus*, *Pasteuria penetrans* and mycorrhizae in plastic bags.
9. Introduce bio agents in to the planting pits while planting in the main field.
10. Apply phorate @3g a.i./plant to inter crops such as banana, black pepper and arecanut in June-July and October-November.

## Methods of diagnosis

### Sampling

Soil and root samples for detection of *R. similis* should be collected when maximum populations of the nematode occur (October-November in India). Maximum populations of *R. similis* are found on coconut at a distance of 100 cm from the bole of the palm and at a depth of 50-100 cm. Fifty g of tender, creamy-white to orange coloured, semi-hard, main roots (about one cm diameter) showing lesions and rotting should be collected to obtain live populations in large numbers (Koshy *et al.*, 1975).

### Extraction

The semi-hard, orange coloured, main root bits are peeled and sliced longitudinally into four to eight pieces of three to five cm length. These sliced root bits are submerged in water contained in Petri dishes or shallow pans at a temperature of 20-25°C, is ideal for extraction of live nematodes from polyphenol rich coconut roots (Koshy *et al.*, 1975). After every 24 hours of incubation,

the water needs to be changed; 50% of the population is extracted after 72 hours. Most of the nematodes are recovered within four to seven days.

#### **Determination of populations and crop loss**

Nematode populations in the tender portions of the main roots can be estimated by staining and blending. Roots may be cut into two cm. long pieces, sliced longitudinally into eight sections and then stained.

#### **Nematodes for the control of pests**

Entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* spp. were isolated from soil around coconut in Kerala which were used for IPM schedule for the control of Rhinoceros beetle, *Oryctes rhinoceros*, L and red weevil, *Rhynchophorus ferrugineus* F. (Sosamma & Banu 1996; Banu *et al.*, 1998; Sosamma *et al.*, 2000; Sosamma 2003).

#### **Conclusion and future prospects**

The burrowing nematode, *Radopholus similis* is second in importance to the red ring nematode, *Bursaphelenchus cocophilus* on the basis of its damage potential on coconut. Though the nematode has been reported in association with various coconut diseases (Govindankutty & Koshy, 1979), no detailed investigations seem to have been carried out anywhere else except India. Screening for resistance/tolerance to *R. similis* in coconut cultivars and their hybrids have indicated the availability of possible resistance in some cultivars. Though breeding in coconut is a long-term process, this area could be profitably exploited. Developing an integrated management schedule for the coconut based on subsistence farming systems involving susceptible perennial crops like arecanut, black pepper, cacao, banana, etc., should be the priority area of research.

#### **Arecanut**

Arecanut or betel nut, *Areca catechu* L., occurs in the humid regions of Asia and the Malay Islands. It is a masticatory of great antiquity and betel chewing is a habit of nearly one-third of the world's population. The ripe fruits are sometimes used as an anthelmintic and astringent in Europe.

#### **Nematodes of Arecanut**

A number of nematodes have been reported from the rhizosphere of arecanut (Nair, 1964; Weischer, 1967; Pizarro, 1969; Koshy *et al.*, 1976, 1978; Reddy, 1978; Koshy *et al.*, 1981; Sundararaju & Koshy, 1982a; Sundararaju *et al.*, 1984; Rama, 1987; Dasgupta & Rama, 1987; Subramaniyan *et al.*, 1988; but only *Radopholus similis* is known to be an important parasite of the palm. A number of other palms have been reported as hosts of *R. similis* and it would not be unexpected if nematode problems with some of these other palms became apparent in the years ahead.

#### ***Radopholus similis***

The burrowing nematode, *R. similis* was first reported from soil around roots of arecanut palm in Mysore, India by Kumar *et al.* (1971) and later by Koshy *et al.* (1975, 1976; Sosamma, 1998b).

#### **Symptoms of damage**

The most conspicuous symptoms of *R. similis* infestation are the appearance of lesions and rotting of roots. The nematode produces small, elongate, orange-coloured lesions on the young, succulent, creamy-white to light-orange coloured portion of the main and lateral roots. Subsequently, the adjoining lesions coalesce and cause extensive root rotting. The thick primary roots

produced from the bole region of the palm exhibit large, oval sunken, brown to black lesions, 2 mm to 2 cm in length.

Nematodes occur inter- and intracellularly in the cortex, but do not enter the stelar tissues. Large numbers of nematodes and their eggs are seen in the cavities that develop consequent to nematode feeding in the cortex (Sundararaju, 1984).

### Biology and life cycle

The burrowing nematode takes 25-30 days to complete one life cycle (J2-J2) on arecanut seedlings at a temperature range of 21-31°C under glasshouse conditions. Chromosome studies have recorded the presence of a haploid number of chromosomes ( $n=4$ ) in many isolates of *R. similis* from arecanut roots (Koshy, 1986b). The arecanut isolate of *R. similis* belongs to the banana race (Koshy & Sosamma, 1977) and multiplies well on carrot discs maintained on one per cent water agar (Koshy & Sosamma, 1980).

The population densities of *R. similis* in arecanut fluctuate; maximum population occurs in roots during October to November and minimum during March to June in India. Populations are also known to vary between samples, types of roots, palms, groves and soil types during the same period (Koshy & Sosamma, 1978a).

### Disease complexes

The fungus *Cylindrocarponobtusisporum* is found associated with lesions caused by *R. similis* in arecanut roots. The fungus when introduced three weeks after nematode inoculation caused more damage to plants compared to inoculations with the nematode alone and it inhibited the rate of multiplication of the nematode (Sundararaju & Koshy, 1984, 1987).

### Economic importance and population damage threshold levels

*R. similis* was recorded from 32% of root samples in the three major arecanut growing States in South India with a maximum population of 440 nematodes/g of root. *R. similis* was found in 55, 45, 44, 30 and 11% root samples from plantations intercropped with banana, black pepper, cardamom, coconut and cacao respectively, compared to 25% from plantations monocropped to arecanut (Sundararaju, 1984).

The population damage threshold level on arecanut seedling is 100-nematodes/seedling or one/800 g of laterite soil. The percentage reduction of growth over uninoculated plants at this inoculum level can be 23, 39, 25, 19 and 38% with respect to shoot length, shoot weight, girth at collar region, root length and root weight under pot conditions in laterite soil.

### Control

#### Resistance/tolerance

None of the 46 accessions of arecanut germplasm in the CPCRI germplasm collection is immune or highly resistant to *R. similis*. The cultivars Mangala (VTL-3) and Fiji (VTL-26) are highly susceptible whereas the cultivars Singapore (VTL-17), Solomon Islands - 2 (VTL-18c) and Saigon (VTL-27) are less susceptible to *R. similis*; cultivars Indonesia 6 (VTL-11) Mahuva 8 and Andaman-5 (VTL-29e) are tolerant to *R. similis* (Koshy *et al.*, 1979; Sundararaju & Koshy, 1982b). The cultivar Indonesia-6 (VTL-11) and Singapore (VTL-17) are known to yield 15% more nuts over local South Canara cultivar. Thus, these cultivars could profitably be recommended for *R. similis* infested areas. The hybrid VTL-11 x VTL-17 is highly resistant to *R. similis*.

## Chemical

As arecanut is chewed directly by many consumers, dosage, frequency and time of application of nematicides on arecanut have to be done carefully to avoid residues in the nut.

A pot culture experiment carried out under field conditions revealed that fensulfothion and aldicarb at 1 g a.i./seedling applied thrice a year for three consecutive years in pots gave control of *R. similis* both in soil and roots. Increase in plant growth with regard to shoot length, shoot weight, root length, root weight, number of leaves and collar girth with fensulfothion were 46, 168, 33, 173, 25 and 41% respectively over control plants after three years (Sundararaju & Koshy, 1986a). In a field experiment in India, treatment with fensulfothion at 50 g a.i./palm and aldicarb at 10 g a.i./palm applied during May/June, September/October and December/January for five years resulted in control of *R. similis* and a substantial increase in both number and weight of nuts compared to untreated palms (Sundararaju & Koshy, 1986b). However, the nuts were not analyzed for their residues, if any, and the cost benefit ratio has not been determined.

Field experiments carried out in arecanut monocrop, arecanut + banana and arecanut + banana + pepper to evaluate the efficacy of neem oil cake and phorate singly and in combination for control of *R. similis* in the cropping system. Even though, all the treatments were significantly superior over the untreated control, the best treatment in these experiments was 1.5 g of phorate in combination with 1 kg neem oil cake which controlled the *R. similis* population in arecanut and subsidiary crops very well (Sudha and Sundraraju, 1998)

A pot trial study to evaluate the combined effect of organic amendments and biocontrol agents viz. *P. lilacinus*, *P. penetrans* and Arbuscular Mycorrhizal Fungi (AMF) against *R. similis* infecting arecanut (*Areca catechu* var. Mangala) was conducted using sandy loam soil amended with various organic matter. The organic amendments used were neem and marotti oil cakes, leaves and tender shoots of sunn hemp and *Glyricidia*, vermicompost, cow dung and coir pith. Maximum nematode control (95%) was recorded in soil amended with *Glyricidia* leaves and bio agents. Significant reduction in root lesion index and maximum leaf area was recorded in these plants. Per cent increase in height and root growth was maximum in plants grown in coir pith amended soil with bio agents which was at par with plants grown in soil enriched with *Glyricidia* leaves and bio agents. Decrease in nematode population was at par in all treatments receiving organic amendments and bio agents as well as bio agents alone compared to nematode alone. All the bio agents were re-isolated from all the treated plants even after three years. Though amendment of soil with organic matter in general was found to increase plant growth and reduce nematode population, the differences were not significant compared to introduction of bio agents in the absence of organic amendments (Koshy *et al.*, 1998, 2002).

## Summary of control measures

Control of *R. similis* on arecanut is difficult under the high-density, multispecies, subsistence farming systems involving perennial crops such as coconut, banana, black pepper, betel vine, cardamom and cocoa. Use of nematicides for the control of burrowing nematode on coconut or arecanut may cause problems of residual toxicity. The following

control measures are suggested:- 1) use of nematode-free planting material of arecanut and other intercrops; 2) avoiding *R. similis* susceptible intercrops such as black pepper and banana in infested areas; 3) use of resistant/tolerant cultivars of arecanut, when available, and other crops in farming systems; and 4) apply 5-10 kg green manure preferably *Glyricidia* or *Crotalaria* ; 5) apply 1 kg of neem oil cake/palm/year, and 6) apply phorate @ 1.5g a.i./plant to the root zone of arecanut, banana and black pepper in June-July and October-November in arecanut based farming systems (Koshy, 1994; Sudha & Sundararaju, 1998).

#### Methods of diagnosis

Soil and root samples for detection of *R. similis* should be collected at a distance of 25-75 cm from the bole of the palm at a depth of 25-75 cm when high population densities are present, such as during October/November in India.

The method suggested for extraction of *R. similis* from coconut root can also be adopted for arecanut.

#### Analysis of Nematodes in intercrops of coconut

The farming systems (both irrigated and rain fed conditions) influence the density and frequency of plant parasitic nematodes in different crops in various seasons and soil types. Homestead farming with coconut as pivotal crop, having banana, tapioca, pepper and arecanut as intercrops is the main cropping system in Kerala. Common nematode genera found in this area are polyphagous and hence the crop combinations, varying soil types and different climatic factors are likely to influence the population dynamics of these nematodes. A multistratified random sampling method was adopted in the survey.

When comparing the effects of different crop combinations, periods of observations and types of soil the population of reniform nematode, *Rotylenchulus reniformis* and *Helicotylenchus* sp. were significantly higher in homesteads planted with coconut alone when compared to coconut in different crop combinations prevalent in the area. The different crops available in the homesteads did not significantly influence variation in the distribution of other nematode species associated with coconut though the population showed variations (Sheela *et al.*, 1990).

The population of nematodes especially *R. similis* associated with banana under monocropping or under mixed cropping as well as pepper grown alone or in combinations with other crops did not show statistically significant variations. The population of *R. similis* in different samples was generally higher in August. But the variations were statistically significant in the case of *R. reniformis* and *Helicotylenchus* sp. only relating to the nematodes associated with coconut rhizosphere. In the case of nematodes associated with banana, the population of *R. similis* and *Helicotylenchus* sp. were significantly higher in August than in May and December. The variation in the population of nematodes associated with pepper did not show statistical significance (Sheela, 1995). The mean population of nematodes did not show any definite trend relating to the distribution in the three types of soil viz., red, laterite and sandy. The data relating to the nematodes associated with coconut alone showed significant variation. *Helicotylenchus* sp. and *Tylenchorhynchus* sp. were significantly more in sandy soil than in red soil. But the population in red soil was on par with that of laterite soil. Reddy (1987) also reported that light sandy soil (coarse textured) is generally more favorable to large proportion of nematodes (Sheela *et al.*, 1990).

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# NEMATODE PESTS OF FRUIT CROPS

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India is the second largest producer of fruits next to China producing 44 million tonnes from an area of 3.72 million hectares and accounting for about 8% of world's fruit production. The diverse agro climatic zones in the country are conducive for the cultivation of almost all the fruit crops like mango, banana, citrus, guava, apple, papaya, pineapple and grapes. Besides these, sapota, anona, jackfruit, ber, pomegranate, peach, pear, almond, walnut, apricot and strawberry are also grown extensively.

Despite being the second largest producer of fruits in the world, the per capita production is only about 100g per day in India. A number of factors contribute to this lowered productivity, one of them being the plant parasitic nematodes. These pathogens form an important limiting factor in the production of fruit crops not only in India but also all over the world. Roots damaged by nematodes are inefficient in utilizing moisture and nutrients in the soil resulting in reduced functional metabolism. These deleterious effects on plant growth result in reduced yield and poor quality of fruits. Apart from the direct damage, nematodes also cause diseases of complex nature. Problems like black head toppling disease of banana, slow decline or dieback of citrus, cherry, prune, apple, walnut and other orchard trees were found to be associated with large populations of plant parasitic nematodes. Moreover, nematodes have also been demonstrated to transmit a number of virus diseases.

The major nematode pests of fruit crops include the genera *Radopholus*, *Tylenchulus*, *Rotylenchulus*, *Meloidogyne* and *Pratylenchus*. The avoidable yield loss due to nematodes in fruit crops in India has been reported by different authors. *Radopholus similis* caused about 38 per cent yield loss in banana (Rajagopalan and Naganathan, 1977), *Tylenchulus semipenetrans* 69 per cent in citrus (Baghel and Bhatti, 1983), *Rotylenchulus reniformis* 28 per cent in papaya (Rajendran and Naganathan, 1981) and *Meloidogyne incognita*, 55 per cent in grapevine (Rajagopalan and Naganathan, 1977).

Although Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Uttar Pradesh and Gujarat are the major fruit producing States of the country, Kerala too contributes its share to the total production. The important fruits cultivated in the State include banana, mango, pineapple, papaya and guava.

## Banana

Banana is the major fruit crop in Kerala. Nematodes constitute one of the major limiting factors in the production of the crop. Though more than 134 species of nematodes belonging to 54 genera have been reported in association with banana, *R similis*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus*, *H. dihystra*, *M. incognita*, *M. javanica*, *Heterodera oryzicola*, and *R. reniformis* are the economically important ones in India (Sundararaju and

Sathiyamoorthy, 2000). Of these, the burrowing nematode is the most destructive and widely distributed nematode.

### Nematodes of banana in Kerala

The major nematode pests of banana in Kerala are *R. similis* and *H. oryzae*. Incidentally, the first report of occurrence of *R. similis* on banana from India was made from Kerala (Nair *et al.*, 1966) where an entire crop was damaged and lost due to its attack. Detection of this nematode initiated further investigations on the nematode problems of banana in Kerala. Initially, fifteen localities between Thrissur in the north and Vellayani in the south were surveyed and plant parasitic nematodes belonging to the genera *Radopholus*, *Helicotylenchus*, *Rotylenchulus* and *Criconemoides* were reported (Varghese and Nair 1968). Subsequently, survey of nematodes associated with banana was undertaken by the Banana Research Station, Kannara under the All India Coordinated Fruit Improvement Project in Palakkad, Thrissur, Ernakulam, Idukki, Alleppey, Kollam and Thiruvananthapuram districts revealed the presence of *R. similis*, *P. coffeae*, *H. multicinctus*, *R. reniformis*, *M. incognita*, *Hoplolaimus indicus*, *Merlinus brevidens* and *Tylenchorhynchus* sp. In 1983, infestation by the cyst nematode, *H. oryzae* on banana c.v. Nendran was observed for the first time in the country (Charles and Venkitesan, 1984). Since then, the nematode has become a potential pest in the banana ecosystem of Kerala. In subsequent surveys conducted at Kannara, the nematode was found parasitising banana cultivated in paddy land in several locations in Thrissur, Ernakulam, Kollam, Kozhikode, Malappuram and Palakkad districts (Venkitesan and Charles, 1985). Meanwhile, survey carried out under AICRP on nematode pests through-

out the state to identify the occurrence and distribution of the major nematodes of the crop revealed that *R. similis* was the predominant nematode pest, its population ranging from 10- 292 per 200g soil and 5-242 per 10g root, the average being 17- 60 per 10g root and 20-80 per 200g soil. The frequency of occurrence of the nematode ranged from 35-86 per cent. Infestation of the cyst nematode was heavy in reclaimed paddy lands. The frequency of occurrence of *H. oryzae* ranged from 20-70 per cent. Other nematodes identified included *Helicotylenchulus*, *R. reniformis* and *M. incognita* (KAU, 1997).

Consequent to the wide spread occurrence of the cyst nematode in the State survey was intensified to locate hot spots of the nematode. The areas identified were Padukutty and Pampadumpara in Idukki district, Konni in Pathanamthitta district and Onnamkutty and Kayamkulam in Alappuzha district, Cheemeni and Kanhangad area in Kasaragod, Kakkavayal and Kodenchery in Kannur, Pattambi and Alathur in Palakkad districts respectively (KAU, 2001).

Since multispecies cropping systems predominate in Kerala, studies were undertaken to determine the nematodes associated with banana in different cropping systems like coconut+ banana, coconut+pepper+ banana, coconut+ pepper+ banana+ vegetables and banana alone. Seven genera of plant parasitic nematodes viz., *R. similis*, *H. oryzae*, *H. multicinctus*, *Pratylenchus* sp., *M. incognita*, *H. indicus* and *Criconemoides* sp. were found in the rhizosphere of banana in the various cropping systems. The build up of *R. similis* and *M. incognita* in banana rhizosphere when planted as inter crop in coconut gardens and homesteads were also studied. Population of *R. similis* was maximum in banana grown

as intercrop in coconut+pepper gardens. The studies on the population build up in different soil types and seasons revealed that light coarse textured, sandy loam soil was best suited to *R. similis* multiplication and maximum population was obtained during August/ September months. Population of *H. oryziicola* was high in reclaimed paddy field where banana was raised as pure crop. Severe infestation of *M. incognita* was observed in banana plants planted along with vegetables (Sheela *et al.*, 1990; Sheela 1995 and Jiji *et al.*, 1999).

Studies on the varietal influence on the different nematodes indicated that Nendran, a popular variety in the State was highly susceptible to *H. oryziicola*. The plants raised in the nematode infested field were severely damaged. Palayankodan was less susceptible to the nematode (Jiji, *et al.* 1999). Similarly, population of *R. similis* was high in Red banana and Nendran. Palayankodan, Robusta, and Poovan supported only less population of the nematode. Contrarily, *M. incognita* equally preferred the five varieties. No significant difference was seen in the population of the nematode in the soil and roots of the varieties (Hebsy, *et al.*, 1997).

### Population dynamics

Detailed studies conducted on the fluctuation of plant parasitic nematodes associated with banana revealed differences in the nematode populations between the different soil types, between rainy and dry seasons and between healthy and infested plants. No interaction between these three factors were seen indicating that their effects were independent (Varghese and Nair, 1968). Population of nematodes in soil was more in loamy and sandy soils. Population of nematodes in the roots was significantly more in the dry season than in the rainy season.

Among the six different soil types (forest soil, red soil, alluvial soil, laterite, coastal sandy and sandy loam soils) tested for their influence on the nematode infectivity, pathogenic effect and multiplication on banana, it was found that coastal sandy and sandy loam soil was preferred by *H. oryziicola* than the other types. The red soil type was noted as least influencing the nematode infectivity and pathogenic effect (Charles, 1989).

Experiments carried out under laboratory conditions on the effect of temperature, moisture and pH at three different levels and their combinations on larval emergence of *H. oryziicola* from cysts indicated that 25°C, flooded condition and 6.7 pH combination, influenced maximum larval emergence from cysts (93%) in the shortest period of 50 days whereas 20°C, flooded condition and 6.4 pH resulted in the least larval emergence (29%).

### Pathogenicity and Crop loss

#### *R. similis*

Pathogenicity tests carried out at Banana Research Station, Kannara using graded loads of populations of *R. similis* indicated that growth of the plant was retarded when the population of the nematode exceeded 100 in 10g root. A reduction of 41 per cent in bunch weight was observed when the population of *R. similis* reached 146 per 10g root weight. Studies carried out on the pathogenicity of *R. similis* with three levels of population viz., 1000, 10000 and 100000 at 5 different growth stages of the crop starting 45, 90, 135, 180, and 225 days after planting revealed that the growth of plant was retarded due to nematode attack. Nematode population was found to reach the peak at flowering and got reduced thereafter. The multiplication of nematodes was observed to be inversely proportional to the initial inoculum level. Population of even

1000 nematodes at active growth stage was sufficient to cause severe reduction in the plant growth. It was seen that the plants could withstand the damage caused by their attack 180 days after planting indicating that the plants should be protected from nematode at earlier stage (Satyanarayana, 1982). Charles *et al.* 1985 reported that increased root populations not only reduced the yield directly, but also influenced other characters such as number of hands, fingers etc.

### *H. orydicola*

The pathogenicity test conducted, with an initial inoculum of 100, 200, 400, 800, 1000 and 2000 cysts per plant conclusively proved that the infestation of *H. orydicola* can adversely affect the plant growth characters, namely, height, girth, leaf production, bunch weight and fruit characteristics like number of hands, fingers and even length, girth, volume and curvature of fingers. The nematode attack adversely affected quality of fruit by increasing the acidity and reducing the total sugars. The fresh weight of feeder root was reduced by 86.49 per cent. The bunch weight was reduced between 20.5 to 5.6 per cent by the different initial inoculum levels. Banana was found to be a good host for *H. orydicola* than paddy, promoting its multiplication and build up of heavy population during the cropping period. The nematode attack induced early flowering and bunch maturity in nematode infected plants compared to the normal healthy plants. The initial inoculum of 800-1000 cysts per plant was observed to be the threshold inoculum to cause maximum pathogenic effect on the plant reducing total bunch weight by 37.7 to 56.6 per cent. Correlation studies of 20 characters with initial nematode inoculum revealed a significant negative correlation on plant growth and yield attributes.

Histopathology of the infected roots showed rupturing of cortical cells and formation of syncytial cells with thickened walls in the stellar region. Black discoloration was seen on the nearby cortical cells without any cellular change. Due to the damage of xylem vessels, flow of food materials to the various parts of the plant was suppressed.

The studies on the biology of the nematode clearly showed that the second stage infective larvae invaded roots and developed to maturity for further multiplication. One life cycle was completed in twenty three days. The average production of eggs per individual female adult was 197 in the cyst body and 88 in egg sac (Charles, 1989).

The host range of the nematode was found to be restricted to paddy and banana which are considered as very good hosts. The common weeds in paddy and banana gardens, and in vegetables including cucurbits grown in banana gardens were recorded as non hosts of this cyst nematode (Charles, 1989).

Nematodes along with fungi like *Fusarium*, *Rhizoctonia* sp also caused damage to banana resulting in disease complex (Charles and Venkitesan, 1993).

### Management

Several measures including physical, cultural, host plant resistance, biological, chemical and integrated nematode management have been reported for controlling the nematodes infesting banana (Rajendran, 1979; Vadivelu and Muthukrishnan, 1979; Reddy *et al* 1991, 1996 and 1997). A brief account of the trials on the management of nematodes in Kerala are detailed below.

### Cultural Measures

The different cultural methods known



to suppress nematodes are summer ploughing, organic manuring, destruction of infested residues, green manuring, crop rotation and adjustment of sowing time (Koshy, 1996).

#### (a) Antagonistic plants

A trial to test the effect of raising *Tagetes erecta*, *Crotalaria juncea*, *Alpinia galanga* and *Asparagus racemosus* on plant parasitic nematodes was conducted in comparison with the popular nematicide carbofuran. Raising of *crotalaria* as intercrop resulted in low population of nematode in roots along with better vegetative and yield characters. *Alpinia galanga* increased the nematode population in roots (Charles *et al.* 1985).

#### (b) Organic amendments and green leaves

The efficacy of neem cake in the management of *R.similis*, *Helicotylenchus* sp. and *Pratylenchus* sp. was studied. Application of neem cake @ 400g per plant once at planting and 4 months after planting resulted in reduction in nematode population and increased bunch weight (Nair, 1979).

The effect of organic wastes and green leaves for managing plant parasitic nematodes was studied at the College of Agriculture, Vellayani by conducting two trials. Among the leaves tried (eupatorium, clerodendron, panal, glyricidia) panal leaves @5 kg applied twice (first at planting and second 2 months after planting) effectively protected the crop from the nematode infestation followed by clerodendron leaves. Among the organic wastes such as saw dust, coirdust, paddy husks and cashew shell powder @2kg/plant tested, application of

paddy husk was found to be most effective. (Nair, 1987).

#### Host Resistance

One hundred and twenty five banana cultivars in the germplasm collection of Banana Research Station, Kannara were screened for their susceptibility /tolerance to *R. similis*. The varieties Chinali (AAB), Galanamalu (AAA), Mas (ABB) Erachivazhai (ABB), Padathi (AAB), Peyan(ABB), Alukhel (ABB) and Thellabontha (ABB) were highly susceptible to *R. similis* with a high nematode population ranging from 720 to 1266 numbers per 10g roots. The cultivars Kadali(AA), Padalimoongli(AAB), Kunnan(AAB), Peykunnan(AAB), Ayiranka Poovan (AB), Pisang Seribu(AA) were observed to have low level of population of *R. similis*. Similarly, varieties Mauritius, Binkehel, Kapok, Ayirankapoovan, Basrai and Sirumalai were highly susceptible to *R. reniformis* (Charles *et al.* 1983).

#### Chemical Control

Application of phorate and phenamiphos 2 g ai /per plant, once at planting and again 4 months after planting effectively reduced infestation of *R. similis* and increased yield of banana considerably (Nair, 1979).

Field trial conducted at Banana Research Station, Kannara showed that cowdung slurry dip treatment of banana suckers had no effect in checking nematode infestation whereas paring and parlinage with a sprinkling aldicarb @ 2g a.i per sucker or paring and dipping suckers in 0.1% aldicarb solution for 10 minutes prevented nematode infestation and increased bunch weight (Venkitesan and Charles, 1983).

In another trial, aldicarb, carbofuran and phorate were reported as most effective, when applied at 2g ai /plant three times, at planting, 75 and 165 days after planting (Venkitesan and Charles, 1988).

### Biological control

Not much progress has been made in the identification of effective bioagents for exploitation for the control of the major nematode pests of banana. From initial studies seven nematophagous fungi viz., *Alternaria alternata*, *Drechslera tetramera*, *Trichoderma viride*, *Syncephalastrum racemosum*, *Curvularia lunata*, *Paecilomyces lilacinus* and *Beauveria bassiana* were identified as highly pathogenic to the cyst nematode, *H. oryzaicola* under *in vitro* condition (KAU, 1999).

The efficiency of micro organisms viz., *T. viride* and *Pseudomonas fluorescens* for nematode management was also studied under field conditions. Maximum yield was recorded in the treatment (Paring + hot water treatment + neem cake + (1 kg/plant) + carbofuran @ 16.6 g/plant), which was on par with treatment (*T. viride* @ 2.5 g/plant at the time of planting + 45 DAP in 1 m<sup>2</sup> basin area) and was statistically superior to rest of the treatments. Similar trend was noticed in hand number and fruit number. The nematode population in soil revealed that, all the treatments significantly reduced the nematode population. More than 70 per cent reduction was noticed in *P. fluorescens* application (2.5g) at planting alone and *P. fluorescens* at planting +45 days after planting respectively (Sheela *et al.* 1999).

### Integrated management

Subsequently, attempts were made

to develop practical and cost-effective integrated management strategy including physical, cultural, biological and chemical methods for managing the major nematodes. One of the major components in the integrated management strategies tested was the use of "clean suckers"(nematode free) prepared through paring and hot water treatment. The objective of paring suckers is to eliminate the nematodes from the planting material. Hot water treatment of pared suckers at 55°C for 20 minutes kills the eggs and other immature stages of nematodes present on the surface.

Several trials were conducted at College of Agriculture, Vellayani with sucker treatment (paring+ hot water treatment at 55°C for 20 minutes), spot application of neemcake @1 kg/ plant and carbofuran @ 0.5 g ai/plant and their combinations. In another set of experiments , phenamiphos 1g ai/plant was added to all the treatments in the first set 90 days after planting. (KAU, 1998).

Results of the different trials indicated that when compared to untreated control, sucker treatment, application of neem cake and carbofuran and their combinations were equally effective in reducing the nematode population and increasing the yield of banana (10 to 65 per cent).

Pooled analysis of data of the various trials conducted from 1986 to 1993 at College of Agriculture, Vellayani indicated that sucker treatment together with application of neem cake or carbofuran was very effective in managing nematodes and increasing the yield of banana. (Sheela *et al.*, 1996).

Effect of various treatments on the *R. similis* population and yield of banana (pooled).

Treatments	Nematode population				Yield	
	Six MAP		Final		Bunch wt. kg.	Percentage increase
	S	R	S	R		
T1-Hot water treatment + paring	112.78 (10.62)*	64.16 (8.01)	314.00 (17.72)	55.35 (7.44)	4.61	14.96
T2 - Neemcake 1 kg/plant	92.93 (9.64)	47.47 (6.89)	241.18 (15.53)	42.38 (6.51)	4.89	21.94
T3 - Carbofuran 0.5 g ai/plant	106.09 (10.30)	25.30 (5.03)	206.49 (14.37)	19.62 (4.43)	5.79	44.14
T4 - T1 + T2	61.62 (7.85)	33.29 (5.77)	180.63 (13.44)	26.11 (5.11)	6.17	53.87
T5 - T1 + T3	78.85 (8.88)	27.14 (5.21)	184.42 (13.58)	28.52 (5.34)	5.84	45.64
T6 - T2 + T3	68.23 (8.26)	26.42 (5.14)	167.18 (12.93)	22.47 (4.74)	5.69	41.90
T7 - T1 + T2 + T3	79.74 (8.93)	26.42 (5.14)	218.1 (14.77)	34.46 (5.87)	6.04	50.62
T8 - Control	185.23 (13.61)	99.00 (9.95)	338.93 (16.41)	101.00 (10.05)	4.01	-
CD (0.05)	(2.45)	(2.13)	(2.59)	(1.35)	0.90	

\* Figures in parentheses are values after  $\sqrt{x}$  transformation

The additional income per plant due to various treatments computed revealed that treatment of suckers alone (paring + hot water treatment) gave an additional income of Rs.13 per plant, while addition of neemcake as basal dose (1 kg per plant) increased the income to Rs. 19 per plant; the additional income due to application of chemicals viz., carbofuran and phorate (basal or at 20 DAP) ranged from Rs. 14 to 19.6. The initial elimination of nematodes from the sucker alone can substantially increase yield and fetch an additional income of Rs.13/- as against Rs.19.0 and 19.6 by the addition of chemicals and organic amendments along with sucker treatments. Subsequent studies also revealed that paring alone was sufficient

to control nematode for increasing the yield by 41 per cent.

The same trial when repeated with additional treatments like application of carbofuran @ 0.5 ai per plant (16.6 g formulated product) alone at 20 days after planting (20 DAP), phorate 0.5 g at per plant (5 g formulated product) alone at planting and at 20 DAP indicated that all the treatments were significantly better than untreated control. However, additional application of nematicides 20 days after planting did not have any added effect. Based on the results of the trials, the following package was evolved and recommended for adoption in the State (KAU, 1999).

Treatment of suckers alone or treatment of suckers + neemcake application can be recommended as an ecofriendly nematode management strategy for banana (KAU, 2002).

### Citrus

Citrus is an important horticultural crop widely grown in tropical and sub-tropical climate. Over 200 nematode species belonging to 44 genera have so far been reported from citrus rhizosphere throughout the world. Eventhough migratory (lesion and burrowing nematodes) and sedentary (root knot nematodes) endoparasites and a number of ectoparasites have been reported from the rhizosphere of citrus, *Tylenchulus semipenetrans* is the only nematode which causes substantial damage to the crop. The discovery of the nematode in 1912 (Thomas, 1913) accelerated the research in fruit nematology. Siddiqi (1961) reported the citrus nematode for the first time from India.

In India it occurs in all citrus growing areas ranging from sub-tropical hilly tracts to tropical areas. With the exception of reports on the association of *T. semipenetrans* with the decline symptoms observed in the citrus plantations in the high ranges of Kerala in the 1960's, the nematode is not of serious concern in the State. Only few studies have been conducted on the nematode pests of citrus.

### Pineapple

More than 100 species of plant parasitic nematodes have been reported in association with pineapple from all over the world. Most of them are of unknown pathogenicity. The species considered of importance are *M. incognita*, *M. javanica*, *R. reniformis* and *Pratylenchus* spp. Studies conducted in Kerala revealed the prevalence of *R. reniformis* in appreciable numbers in

the rhizosphere of pineapple. The frequency distribution of the nematode ranged from 2-22 per cent in surveys conducted in different locations in Thiruvananthapuram, Kollam, Kottayam, Ernakulam and Idukki districts.

### Papaya

Of the several nematodes reported to be associated with papaya only two genera are considered to be economically significant in the cultivation of papaya. These are *Meloidogyne* sp. and *R. reniformis*. Both these nematodes are the major nematode pests of papaya in Kerala. Besides, *H. multicinctus* was also seen associated with the fruit plant. Survey conducted at Thiruvananthapuram, Kollam, Kottayam and Ernakulam districts recorded the occurrence of *H. multicinctus* and *R. reniformis* and their population ranged from 0-37 and 0-85 per 200g soil. The frequency distribution of the nematodes were 67 and 75 per cent respectively. (KAU, 1987)

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# NEMATODES OF SPICES

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Spices are aromatic substances of plant origin commonly used for flavouring, seasoning and imparting aroma in foodstuffs and are being used by mankind since earliest times. No other commodity has played a more pivotal role in the development of modern civilization as spices. They have greater importance as ingredients in food, medicine, perfumery, cosmetics, toiletries etc. They also possess antioxidant, antimicrobial, pharmaceutical and nutritional properties. Their key role in preserving global health is being unravelled day by day.

ISO document 676 lists 109 herb and spice plants useful as ingredients in food. India is considered as the home of majority of spices from ancient times and it produces a large share of all spices. In 2003-04, it is estimated that India exported about 2,46,566 MT of spices valued at Rs. 1,905 crores (Spices Board, 2004). This is almost 40 per cent of the global trade in spices.

Kerala accounts for a major share of area under spice cultivation. It has got the highest area in black pepper, cardamom and some tree spices. Several biotic and abiotic factors seriously hamper the production of spices crops in Kerala. Although diseases and insect pests have long been recognized as important constraints in spice production, extensive research on plant parasitic nematodes is lacking. Their interaction with other plant pathogens, particularly soil borne fungi, and in many cases the synergism that results in more damage than either pathogen alone is pertinent.

## Black Pepper (*Piper nigrum* L.)

Black pepper, the 'king of spices' is the most important spice crop in the world and is being cultivated in the hot and humid parts of the world. India's production of pepper during 2002-03 was 70,600 t and covered an area of 223,060 ha (Spices Board, 2004). Out of this, Kerala from an area of 208,610 ha produces 67,360 t of black pepper. Black pepper is believed to have originated in the hills of South West India. It has extensive culinary uses for flavouring and preserving processed foods and is important medicinally too.

## Nematodes of Black Pepper

Plant parasitic nematodes are an extremely important limiting factor in black pepper cultivation. The damage due to nematodes is more severe in intensive production systems like black pepper nurseries. Although a sizeable number of plant parasitic nematodes have been recorded on black pepper (Table 1), only burrowing nematodes (*Radopholus similis*) and root knot nematodes (*Meloidogyne* spp.) can be regarded as economically important. The root-knot nematode, *Meloidogyne* sp., was the first nematode to be recorded on black pepper in India. In 1906, Butler reported root-knot nematodes from black pepper in Wynad, Kerala (India). Subsequently, *Meloidogyne javanica* and *M. incognita* have also been reported from India, and other countries (Sundararaju *et al.*, 1979a, Ramana & Mohandas, 1983). A new species, *M. piperi* has been recently described from Kerala, India (Sahoo *et al.*, 2000).

Intensive surveys have shown that almost 70% of black pepper gardens in Kerala and Karnataka are infected with nematodes and the occurrence of root knot nematode was maximum (69.8 %) in these gardens, followed by burrowing nematode (57.5 %). However, the most damaging nematode of black pepper is the burrowing nematode. Association of the *R. similis* with the yellows disease of pepper was first reported as early as in 1936 and has been detected in several pepper growing countries, including Indonesia, Malaysia, Thailand and Sri Lanka (Van der Vecht, 1950; Christie, 1957; 1959; Sher *et al.*, 1969; Reddy, 1977; Gnanapragasarn *et al.*, 1985). Subsequently, *R. similis* was reported from black pepper from India (D'Souza *et al.*, 1970; Kumar *et al.*, 1971; Venkitesan, 1972; Koshy *et al.*, 1978; Ramana *et al.* 1987a; Mohandas & Ramana, 1987c; Ramana & Mohandas, 1989).

Another nematode of wide occurrence in black pepper tracts is *Trophotylenchulus piperis*. It has been reported on black pepper roots collected from several parts of Kerala and Karnataka (Mohandas & Ramana, 1982; Mohandas *et al.*, 1985; Ramana & Mohandas, 1987a, 1989; Sunderaraju *et al.*, 1997). *T. piperis* completed its life cycle on black pepper roots within 55 days at a room temperature of 24-32°C (Sunderaraju *et al.*, 1995). Feeding of this nematode on black pepper roots, though caused drying and shrinkage of cells in the vicinity of infection it is considered as a weak parasite (Ramana & Eapen, 1997).

Studies in India showed that high populations of *R. similis* occurred more frequently in black pepper plants affected with slow-decline disease than in healthy plants, though combined infection with root-knot

nematodes is very common. Discriminate analysis indicated the positive involvement of *R. similis* in this disease (Ramana *et al.*, 1987a). The nematode penetrates roots within 24 hours of inoculation and the cells around the site of penetration become brown (Venkitesan & Setty, 1977). It completes its life cycle within 25 days, at a temperature range of 25-28°C (Geetha, 1991). The black pepper isolate of the nematode is easily cultured on carrot discs at 25°C (Koshy, 1986b), has a haploid number (n=4) of four chromosomes and belongs to the 'banana race' (Koshy, 1986b; Jasy, 1991; Ramana, 1992).

In Kerala, *R. similis* population in roots of pepper occurs during September-October and minimum during April-May (Ramana, 1986; Mohandas & Ramana, 1988). A low soil temperature coupled with adequate soil moisture and availability of fresh tender roots help in the build up of its population during September-October. *M. incognita* population in black pepper gardens reaches maximum during April/May and minimum during December/January (Ramana, 1992).

#### *Economic importance*

Nematodes are involved in 'slow decline (slow-wilt)' disease of black pepper in India, which is almost identical to 'pepper yellows' in Indonesia (Butler, 1906; Van der Vecht 1950; Mohandas & Ramana, 1987b). Black pepper was introduced to Indonesia from Kerala, India (Nambiar, 1977) and it is quite likely that the burrowing nematode was also introduced along with the rooted cuttings of black pepper. The slow-wilt disease was first reported from Wynad area in Kerala as early as 1902 and Krishna Menon (1949) reported mortality up to 10% of the vines due to the disease.



In Kerala, more than 90% of black pepper gardens are having root-knot nematode infestation (Ramana *et al.*, 1987a; Ramana & Mohandas, 1987b). A population level of 250 burrowing nematodes/g of roots was constantly recorded in slow-decline affected pepper vines in Kerala (Ramana, 1986). In pathogenicity tests, *R. similis* caused significant reduction in the growth and yield of black pepper (Mohandas & Ramana, 1991). Black pepper vines of any age group are susceptible to this nematode (Ramana, 1992). An initial population of ten root-knot nematode juveniles per rooted cutting reduced growth by 16%, while, a maximum of 50% reduction was observed at an inoculum level of 100,000 over a period of one year in sterile soil under potted conditions (Koshy *et al.*, 1979b). *M. incognita* was found highly pathogenic at 100-10,000 juveniles per seedling (Mohandas & Ramana, 1991).

Studies under simulated field conditions showed that *R. similis* and *Phytophthora capsici* alone or in association resulted in root rotting leading to slow decline disease (Ramana *et al.*, 1992). Black pepper plants showed wilting symptoms quicker when root-knot and burrowing nematodes were inoculated first followed by *P. capsici* (Ramana *et al.*, 1992; Anandaraj *et al.*, 1996a, 1996b). The root gall development and population build up of *M. incognita* was suppressed in black pepper on inoculation with *R. similis* in succession in sterile soil under pot conditions (Sheela & Venkitesan, 1981). Synergistic interaction has been noticed between *M. incognita* and *Fusarium* sp. too (Sheela & Venkitesan, 1990).

#### **Symptoms of damage**

The primary symptom of the slow decline (yellows) disease is the appearance of pale yellow or whitish yellow drooping

leaves on the vines. Leaves of vines infested with *Meloidogyne* spp. exhibit dense yellowish discolouration of the interveinal areas making the leaf veins quite prominent with a deep green colour, whereas leaves of the vines infested with *R. similis* show uniform pale yellow or whitish discolouration and typical drooping (Ramana *et al.*, 1994). Yellowing and shedding of leaves, cessation of growth and dieback symptoms are very commonly seen in such vines. These symptoms are well pronounced when soil moisture is depleted. The vines die within three to five years of initiation of yellowing and hence the name "slow-decline or slow wilt" disease. In bearing vines, shedding of spikes (inflorescences) is a major symptom. In large plantations, nematode affected areas become conspicuous as yellow patches that later turn as barren standards that have lost their vines, or standards supporting dead vines without any leaves.

The tender thin, white, feeding roots show typical orange to purple coloured lesions, characteristic of *R. similis* infestation. Lesions are not clearly seen on older roots, being brown in colour. The root system exhibits extensive rotting and the main roots are devoid of fine feeder roots that rot quickly. Extensive necrosis of larger lateral roots develops subsequently. Root systems become heavily galled in the case of root-knot infestation and the adult females with egg masses are generally enclosed deep within the roots (Ramana, 1992; Ramana *et al.*, 1994). The galls are smooth and bigger in size in a few cultivars but small galls with exposed egg masses giving a pitted rough appearance in majority of cultivars.

#### **Management measures**

No effective control measures are available at present to control slow-decline

disease. Being a perennial crop trailed on live standards, any control measures need to be repeated every year under Indian conditions. The perennial multi-cropping systems are ideal situations where the burrowing nematode multiplies and causes heavy damage to all the susceptible crops. The high-density multi-species cropping pattern does not permit use of nematicides, as most of the crops are export-oriented and some products are consumed without any processing or cooking. The situation is further complicated where arecanut and coconut are used as live standards as they are very good hosts of *R. similis*.

**Cultural practices:** Mulching and bio-fumigation ameliorate symptoms of slow-decline/pepper yellows. Addition of chopped leaves of *Glyricidia maculata* (10g/kg soil) as green manure reduced populations of *R. similis* and increased the plant growth (Jasy & Koshy, 1992). Application of botanicals like neem oil cake reduced root-knot nematodes (Ramana *et al.*, 1992). Interestingly, foliar yellowing and necrosis of slow-decline affected vines in Kerala were attributed to N and K deficiencies (Wahid *et al.*, 1982).

Among the large number of live standards used in black pepper gardens, coconut and arecanut are good hosts of *R. similis* while several of these are highly susceptible to root knot nematodes too. However, *Garuga pinnata* Roxb. and *Macaranga indica* Wight are not susceptible to root knot nematodes and the popular live standards, *Erythrina indica* Lank. and *Gliricidia sepium* (Jacq.) Walp. are less susceptible. Several inter crops like banana, ginger and turmeric and a large number of weeds are also susceptible to these nematodes (Koshy *et al.*, 1977; Ramana, 1986). Large numbers of

weeds that are found in pepper gardens have been recorded as hosts of the root-knot nematodes.

**Resistance and tolerance:** A number of black pepper germplasm accessions, including wild types, were screened against *M. incognita* and *R. similis* by several workers (Venkitesan & Setty, 1978; Koshy & Sundararaju, 1979; Jacob & Kuriyan, 1979a; Mohandas & Ramana, 1983; Ramana & Mohandas, 1986, 1987b; Koshy, 1987b). Wild collection Vittal No. 430, *Piper hymenophyllum* and *P. attenuatum*, recorded least (less than 30%) root reduction and minimum reproduction of *R. similis*. However, a local cultivar at Peringamala, Kerala, India was not invaded by *R. similis* (Jacob & Kuriyan, 1979b). No resistance or tolerance was found to the nematode on screening a number of cultivated and wild germplasm, intercultivar hybrids and open pollinated seedlings, except for *P. colubrinum*, which is now widely used as a root-stock to graft cultivated pepper plants (Ramana *et al.*, 1987b; Ramana, 1992). A total of 101 cultivars, 74 accessions of wild *Piper* sp. and 140 inter cultivar hybrids, were screened against *M. incognita* of which one cultivar, CLT-P-812 was found resistant (Ramana & Mohandas, 1986, 1987b). This accession was later released as 'Pournami' for cultivation in root knot infested areas (Ravindran *et al.*, 1992). The biochemical changes in this variety consequent to nematode infection have been studied (Eapen *et al.*, 1999). Some of the wild related species of *Piper* are also resistant to root knot nematodes (Ramana, 1992).

**Chemical control:** Root-knot infestation in black pepper nurseries has been a serious problem in several nurseries of Kerala. Fumigation of nursery potting mixture was

effective in checking the infestation (CPCRI, 1974, Koshy, 1986a; Mohandas & Ramana, 1987a). A number of pesticides have been found effective in reducing nematode populations on black pepper in pot trials as well as in preliminary field trials. Aldicarb sulphone at 8 kg a.i./ha was most effective for control of *R. similis* on pepper in pot trials (Venkitesan, 1976; Venkitesan & Setty, 1979). On integrating fertilizers (N=100g, P=40g, K=140g/vine in two equal split doses) with aldicarb at 1 g a.i./vine applied twice a year (May/June and October/November) plus earthing up to 50 cm radius at the base of the vines and mulching the vine base with leaves, there was 83% reduction in foliar yellowing and 33-88% reduction in *M. incognita* juvenile populations (Venkitesan & Jacob, 1985). Aldicarb/carbofuran/phorate at 3 g a.i./vine applied in May/June and again in September/October resulted in the remission of foliar yellowing and reduction in nematode populations. Among the above three nematicides, phorate was superior (Ramana, 1986; Mohandas & Ramana, 1987a; Lokesh & Gangadharappa, 1995; Sundararaju & Sudha, 1998). The chances of rehabilitating the severely affected vines by application of nematicides are slim because of the heavy damage already caused to the root system and the inability of such plants to put out fresh roots for quick rejuvenation.

**Biological control:** By fortifying potting mixture with biological control agents, nematode-free cuttings can be raised. A number of organisms have been tested and found effective in suppressing root knot nematodes. Promising among these are *Paecilomyces lilacinus* (Ramana, 1994; Sosamma & Koshy, 1997), *Pochonia chlamydosporia* (= *Verticillium chlamydosporium*) (Sreeja et al., 1996),

*Pasteuria penetrans* (Sosamma & Koshy, 1997), *Bacillus* spp. (Sheela et al., 1993) and *Pseudomonas fluorescens* (Eapen et al., 1997). A number of rhizobacteria that are antagonistic to root-knot nematodes have been isolated recently (Beena et al., 2001). Black pepper plants preinoculated with vesicular arbuscular mycorrhizal fungi like *Glomus fasciculatum*, *G. etunicatum*, *G. mossae* and *Gigaspora margarita* recorded a significant increase in growth even in the presence of root-knot nematodes (Anandaraj et al., 1991; Sivaprasad et al., 1990, 1992). However, there are only very few successful attempts to control *R. similis* by using any of the fungal bioagents, probably due to the migratory endoparasitic nature of this nematode (Geetha, 1991; Ramana, 1994). The mycorrhizal fungus, *Glomus fasciculatum* suppressed burrowing nematode infestation (Anandaraj et al., 1996c). Recently, rhizobacteria that suppressed *R. similis* infesting black pepper were identified in greenhouse studies (Beena et al., 2003). Endophytic bacteria that suppress root-knot nematodes also have been isolated from black pepper (Aravind et al., 2004).

#### *Future prospects*

Nematodes will continue to be a major production constraint, as long adequate attention is not given for their effective management. Developing cropping systems, avoiding susceptible live supports or standards, incorporating an integrated nematode management system with minimum or no nematicide application, should be the main thrust of research to increase black pepper yield in areas infested with damaging nematodes. Nurseries should be the target sites for nematode management. Exploitation of the wide variability available in black pepper to nematodes, particularly *R. similis*, should be a major priority.

## **Cardamom (*Elettaria cardamomum* Maton)**

Cardamom, known as the “Queen of spices”, has its origin in the evergreen rain forests of South India. It is basically a shade loving plant. India and Guatemala are the main producers and exporters of cardamom. Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Kampuchea and Papua New Guinea are also cardamom growers. The area under cardamom cultivation in India during 2003-04 was 73,237 ha and the total production was 11,580 t (Spices Board, 2004). Out of this, Kerala accounts for the highest area (56.4%) and production (76.6%). Cardamom is used in food, perfumery, liquor and pharmaceuticals as a flavour and carminative.

### ***Nematodes of cardamom***

Nematological investigations on cardamom have been undertaken in India, where a number of plant parasitic nematodes have been found (Table 1). The most important nematode problem is caused by the root-knot nematodes, *Meloidogyne* spp., although the lesion nematode *Pratylenchus coffeae* and the burrowing nematode, *R. similis* are known to cause root rotting (D’Souza *et al.*, 1970; Kumar *et al.*, 1971; Khan & Nanjappa, 1972; Viswanathan *et al.*, 1974; Sundararaju *et al.*, 1979b). Reniform nematode, *Rotylenchulus reniformis*, is also recorded on cardamom (Eapen, 1995a).

Widespread occurrence of root-knot nematodes, *Meloidogyne incognita* and *M. javanica* has been reported in cardamom nurseries and plantations in India (Kumar *et al.*, 1971; Koshy *et al.*, 1976; Ali & Koshy, 1982a; Ali, 1985, 1986a; Raut & Pande, 1986). Root knot nematode population dynamics in cardamom plantations is influenced by rainfall, soil moisture and

temperature, and crop phenology. As a result, the root knot nematode population is generally high during the post monsoon period (November-January) (Eapen, 1993). The nematodes are disseminated through infested seedlings and rhizomes used for propagation.

### ***Economic importance***

Root knot nematodes are very serious in nurseries than in adult plantations. In primary nurseries, more than 50% of the germinating seeds do not emerge due to infection of the radicle and plumule by the second stage juveniles of the root-knot nematode. Up to 40% of such seedlings do not establish in the secondary nursery. Young seedlings are more susceptible to root knot nematode attack than mature plants and galling is more prominent in seedlings (Eapen, 1992).

The heavily shaded, hot, humid atmosphere and continuous availability of soil moisture prevalent in cardamom plantations are congenial conditions for the multiplication of root-knot nematodes. A yield loss of 32-47% has been reported due to root knot nematode infestation from the results of a nematicide experiment (Ali, 1985, 1986b). Microplot studies under simulated field conditions showed 46.6% yield loss at an initial inoculum level of 4 nematodes/100 cc soil (Eapen, 1987; Eapen, 1994). The incidence of rhizome rot and damping-off diseases caused by the fungus *Rhizoctonia solani* increases in the presence of *M. incognita* in the nurseries (Ali, 1986b; Eapen, 1987; Ali & Venugopal, 1992; 1993). Root knot nematode population was 5-10 more in ‘katte’ (a virus disease) affected cardamom plants (Ali, 1989).

### ***Symptoms of damage***

The infested cardamom seedlings at the

two-leaf stage show marginal yellowing and drying of leaves and severe galling of roots. On transplantation to a secondary nursery, they exhibit curling of the unopened leaves. These leaves mostly emerge after the breaking open of the pseudostem. In secondary nurseries, the infested plants are stunted and yellowed with poor tillering, drying of leaf-tips and margins, and heavy galling of root (Ali & Koshy, 1982a; Eapen, 1995b). Patches of stunted and weak plants are a common symptom of nematode infestation in cardamom plantations (Eapen, 1994; 1995b). Heavy root-knot nematode infestation in mature plants in a plantation causes stunting, reduced tillering, yellowing, premature drying of leaf-tips and margins, narrowing of leaf blades, a delay in flowering, immature fruit-drop and reduction in yield. Though galling of roots is not prominent, the infested roots exhibit a "witches broom" type of excessive branching.

#### **Management measures**

Even though there is a general awareness about nematodes infesting cardamom, the recommended control measures are not being followed by many growers.

**Cultural control:** It is advisable to change nursery sites every year, but this is not always practicable in view of the difficulties involved in getting suitable sites having facilities for irrigation. Hence, disinfection of the nursery beds needs to be carried out every year. Disinfection of nursery beds with methyl bromide at 500 g/10m<sup>2</sup> is effective in controlling root-knot infestation in both primary and secondary nurseries (Ali & Koshy, 1982b).

A large number of annual weeds present in the cardamom plantations and the common shade trees, *Erythrina indica* and

*E. lithosperma*, are hosts of root-knot and help in the build up of nematode populations (Muniappan, 1993). No resistance to root-knot nematodes has been found and the popular cardamom cultivars, Malabar, Mysore and Vazhuka are all susceptible (Hegde *et al.*, 1993; Eapen, 1995b).

**Chemical control:** It has been demonstrated that application of aldicarb at 5 kg a.i./ha three times, every three months, results in increased growth and vigour of seedlings both in primary and secondary nurseries (Koshy *et al.*, 1979a; Jacob & Chandrasekharan, 1984; Ali, 1986b, 1987). Drenching of nursery beds with fenamiphos also significantly reduced root knot nematodes (Ali, 1986c). Aldicarb, carbofuran, phorate at 5, 10 or 15 kg a.i./ha respectively, have been applied in primary nurseries of cardamom for control of *M. incognita*. None of the nematicide treatments totally prevented nematode infestation but there was significant reduction in root-knot densities. Aldicarb at the very high level of 15 kg a.i./ha reduced nematode numbers by 90% (Ali, 1987). Application of aldicarb/carbofuran/phorate at 5g and 10g a.i./plant and neem oil cake at 500g and 1000g/plant twice a year increases yield of cardamom plants infested with *M. incognita* from 47 to 88%. Maximum yield was obtained from the plants receiving neem oil cake at a rate of 1000g/plant followed by 500g/plant (Ali, 1984). However, in another study, application of phorate @ 2.5-5.0 g a.i./plant reduced the nematode population and increased the yield by more than 40% (Eapen, 1995b).

**Biological control:** Cardamom nurseries are ideal for practicing biological control. There are reports that *Gigaspora margarita* and *Glomus fasciculatum* suppressed *M. incognita* infestation and

enhanced growth and vigour of seedlings (Thomas *et al.*, 1989). *P. lilacinus* reduced root-knot nematodes by 48.5% to 57% in pot culture studies and by 19.7% in field studies (Eapen, 1995b; Eapen & Venugopal, 1995). Some native isolates of *Trichoderma harzianum* and other *Trichoderma* spp. are potential antagonists of root knot nematodes. Suppression of root-knot nematodes by this fungus has been clearly proved in laboratory, greenhouse and also in cardamom nurseries (Eapen *et al.*, 2000).

### **Future Prospects**

Nematode management in cardamom nurseries has to be given top priority. Highly toxic fumigants and granular nematicides are unlikely to be used for this. More target specific means of nematode management like use of endophytic and rhizospheric bacteria and fungi have to be developed to control nematodes in nurseries. In plantations, the general soil health can be sustained by suitable soil amendments.

### **Ginger (*Zingiber officinale* Rosc.)**

Ginger is an herbaceous perennial belonging to the family Zingiberaceae. Ginger originated in South-East Asia, most probably in India. It is grown in many countries of the tropics and subtropics and is used widely in food, beverages, confectionery and medicines. India is the largest producer of dry ginger in the world contributing about 30% of the world's production. In India the total area under cultivation during 2002-03 was 85,930 ha and the total production was 307,370 t (Spices Board, 2004). However, ginger cultivation in Kerala is confined to certain pockets comprising of about 9,000 ha producing only about 10.5% of India's production. Seed rhizomes are used for propagation of ginger, which is usually grown as an annual in

cultivation. It is grown either as a monocrop or as an intercrop in many farming systems.

### **Nematodes of ginger**

Plant parasitic nematodes belonging to 17 genera were reported on ginger (Sundararaju *et al.*, 1979b, Rama & Dasgupta, 1985; Kaur, 1987; Ramana and Eapen, 1998) the most important parasites are *Meloidogyne* spp., *R. similis* and *P. coffeae*. In Kerala, *M. incognita*, *R. similis* and *Pratylenchus* spp. were the major nematodes species found in the rhizosphere of ginger (Mammen, 1973; Charles, 1978; Charles & Kuriyan, 1982; Sheela *et al.*, 1995). Several species of root knot nematodes viz. *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* have been reported as parasites of ginger in various countries. Other nematodes observed include *R. reniformis*, *H. multisinctus*, *Tylenchorynchus* spp., *Hoplolaimus indicus*, *Criconeoides* spp. and *Xiphenema* sp. (Sheela *et al.*, 1995).

### **Economic importance**

In Kerala, *M. incognita* is widely distributed in ginger fields and causes a loss of 46.4% (Charles, 1978). A reduction of 74% rhizome weight has been recorded with an initial inoculum level of 10,000 root-knot nematodes per plant over a period of six months under potted conditions (Sudha & Sundararaju, 1986). In another study on ginger, an avoidable yield loss of 43 % was observed at an initial population level of 166 *M. incognita* larvae per 250 g soil (Sheela *et al.*, 1995). *M. incognita* significantly reduced the height, root biomass and yield of ginger at an initial inoculum level of 02.2 to 200 J2/100 cc soil (Ramana & Eapen, 2001). In the case of *R. similis*, an initial inoculum level of 10,000 nematodes per plant has been reported to cause 74% reduction in rhizome weight and an initial inoculum level of ten

nematodes per plant reduced shoot weight, root weight and rhizome weight by 43, 56 and 40%, respectively, in a pot experiment (Sundararaju *et al.*, 1979c).

Incidence of rhizome rot of ginger caused by *Pythium aphanidermatum* is reported to be severe when rhizomes are infested with nematodes. Recent studies have shown that, ginger plants inoculated with root knot nematodes developed disease symptoms early on inoculating with *P. aphanidermatum* (Ramana *et al.*, 1998). However, bacterial wilt of ginger caused by *Ralstonia solanacearum* is not influenced by nematode infection.

### *Symptoms of damage*

The root-knot nematodes cause typical galling and knotting of roots. In addition, nematodes parasitizing the rhizomes produce galls in the outer tissues, which may turn to necrotic spots subsequently. The second stage juveniles of *M. incognita* invade the rhizome through the axils of leaf sheaths. In fibrous roots, penetration occurs in the area of differentiation and galls are formed. In fleshy roots, the entire length of root is invaded (Parihar & Yadav, 1986). The nematode produced giant cells with thickened cell walls on vascular tissues in the fleshy roots and rhizomes (Routaray *et al.*, 1987a). The fibrous roots were very much reduced. Infested rhizomes have brown, water-soaked areas in the outer tissues, particularly in the angles between shoots. Nematodes continue to develop after the crop has matured and been harvested and induce breakdown of the seed rhizomes.

Heavily infested plants show external symptoms like stunting, poor tillering and chlorotic leaves with marginal necrosis. The affected ginger plants mature, dry faster and

die prematurely than healthy ones, leaving a poor crop stand at harvest. Infested rhizomes serve as a source of infection and means of dissemination. *P. coffeae* is reported to cause 'ginger yellows' disease, prevalent in Himachal Pradesh and some North-Eastern States (Kaur & Sharma, 1990). The nematode infestation caused yellowing of leaves and dry rot like symptoms with dark, brown necrotic lesions on rhizomes. The dry rot symptoms caused by *Pratylenchus* spp. are indistinguishable from those caused by some fungi.

### *Management measures*

Being an export-oriented crop, which is occasionally consumed raw, nematodes of ginger have to be managed in an eco-friendly manner. Shifting cultivation is common in the case of ginger and it checks nematode problems to some extent. A careful blend of the following measures may provide adequate management of the nematode problems in these crops.

Cultural control: Nematodes surviving in stored seed rhizomes will be disseminated through propagative material. Therefore, use of seed rhizomes that are free of nematodes is an effective means of controlling or reducing damage by nematodes. Nematode-free planting materials should be selected from fields of known history. Hot water treatment of seed rhizomes at 50-55°C for 10 minutes or rhizome solarization was found to reduce the initial load of nematodes and other pathogens in ginger (Colbran & Davis, 1969; Anonymous, 1971; Kumar *et al.*, 2005).

Effect of mulching or applying well decomposed cattle manure or poultry manure or compost or neem oil cake on nematode build up in ginger was studied by several

workers (Colbran, 1974; Kaur, 1987; Stirling, 1989; Mohanty *et al.*, 1995; Dohroo *et al.*, 1994; Vadhera *et al.*, 1998b). Ginger plots mulched with mahaneem leaves @ 2.5kg/m<sup>2</sup> reduced root knot nematode population (Das 1999). Inter cropping bell pepper with ginger significantly reduced both *P. penetrans* and *M. incognita* and improve the yield of ginger (Sharma & Bajaj, 1998). Incorporation of organic materials fortified with biocontrol agents like *Trichoderma* spp., *P. lilacinus*, *V. chlamydosporium* etc. is another excellent option to prevent the nematode build up (Eapen & Ramana, 1996). Organic amendments not only decrease the nematode population densities but also improve soil structure and water-holding capacity, reduce other diseases and limit weed growth.

Most of the weeds that are present in ginger growing areas are known hosts of root-knot nematodes. A clean cultivation can reduce the nematode problems. Crop rotations can be easily standardized for endemic areas of various nematodes. When ginger is cultivated as an intercrop, host status of the component crops to be taken into account.

**Physical control:** Soil solarization and rhizome solarization are two technologies that are successful in minimizing soil-borne and seed-borne disease problems in ginger (Balakrishnan *et al.*, 1993; Kumar *et al.*, 2005). These technologies are useful to minimize the initial nematode problems. Soil solarization coupled with bio-enhancement with useful microorganisms will provide sustained nematode control in ginger fields. Following, frequent soil tillage during summer period will result in significant reductions in nematode populations.

**Host resistance:** The use of resistant cultivars is an elegant, economical and

environmentally safe method for controlling root-knot nematodes. In ginger, there are very few reports of nematode resistant cultivars or varieties. In a preliminary evaluation, a few lines of ginger (Acc. Nos. 36, 59 and 221) were found resistant to *M. incognita* (Eapen *et al.*, 1999). One of these has been recommended for release as 'IISR Mahima' (Sasikumar *et al.*, 2003).

**Chemical control:** Soil fumigation or application of granular pesticides or dip treatment though recommended for control of ginger nematodes in other countries, in India, there are very few reports of evaluating some pesticides for controlling nematodes (Kaur, 1987; Mohanty *et al.*, 1995). In Kerala, application of carbofuran @ 3.3g/m<sup>2</sup> or neem cake (200g/m<sup>2</sup>) at the time of planting is recommended for the control of nematodes associated with ginger. In endemic areas application of neem cake (100g/m<sup>2</sup>) 45 days after planting is also recommended (KAU, 2002) However, Nematicides application is not advisable for a crop like ginger.

**Biological control:** A large number of bacterial and fungal isolates of biocontrol agents were isolated from ginger fields through random surveys (Ramana *et al.*, 2002). Many of the fungal isolates parasitized root knot nematode egg masses and suppressed their egg hatching. Toxic metabolites of some of them caused mortality of second stage juveniles in addition to direct parasitization. These studies indicated that five biocontrol agents namely, *V. chlamydosporium*, *P. lilacinus*, *Fusarium* sp., *Aspergillus nidulans* and *Scopuloriopsis* sp. suppressed root knot nematode population significantly (Eapen *et al.*, 2005).

#### **Future prospects**

Area under ginger cultivation is increasing in India by extending cultivation to newer



and unconventional regions. Fresh surveys have to be taken up in most of these ginger-growing areas for obtaining a better picture of the economic importance and distribution of these nematodes. Effective awareness programmes have to be launched among the farming community for imparting a clear understanding of nematode damage. Considering the non-availability of new pesticides, cost-effective biological formulations coupled with soil/rhizome solarization have to be deployed and promoted for early root protection against nematode attack.

### **Turmeric (*Curcuma longa* L.)**

Turmeric of commerce is the dried and processed rhizomes of the plant *Curcuma longa* L. It is best known as a condiment although the plant has uses in medicine, many socio-religious functions, as a biopesticide and also as a natural colouring agent. Though it is reported to have originated in South-East Asia, the rich biodiversity of *Curcuma* genus in India indicates its Indian origin. It is grown mostly in India, and to a small extent in China, Indonesia, Peru and Jamaica. In India, the total area under cultivation during 2002-03 was 149,410 ha with a production figure of 527,960 t (Spices Board, 2002). Kerala's share in this is only 3,140 ha in area and 6,940 t in the total production. It is cultivated either as a monocrop or an intercrop in many farming systems.

It is indispensable in the preparation of curry powder, and is an important source of natural yellow dye. It is also used as a colouring matter in the drug, confectionery and food industries. The rhizomes of *C. aromatica* Salisb., a close relative of *C. longa*, is also a source of turmeric.

### ***Nematodes of turmeric***

A number of species of plant parasitic

nematodes have been reported in association with turmeric in India (Nirula & Kumar, 1963; Sundararaju *et al.*, 1979b; Dasgupta & Rama, 1987; Gunasekharan *et al.*, 1987; Haider *et al.*, 1998a; Rama, 1987; Mani & Prakash, 1992; Routaray *et al.*, 1987b; Bai *et al.*, 1995) of which *Meloidogyne* spp., *R. similis* and *P. coffeae* are of economic importance. Ayyar (1926) first reported root knot nematode infestation in turmeric. Two species of root-knot nematodes, *M. incognita* and *M. javanica* have been reported on turmeric, but most investigations have been concerned with *M. incognita*.

### ***Economic importance***

An avoidable yield loss of 33.6% was reported in turmeric due to root-knot nematode infestation (Ray *et al.*, 1995). Significant reduction in growth and yield of turmeric were noticed in plants inoculated with >1000 root knot nematode juveniles per plant (Sudha *et al.*, 1989). When four varieties of turmeric were tested against *M. incognita*, maximum reduction (18%) of fresh rhizome weight was observed in Suvarna at a Pi=2 juveniles per gram of soil (Ramana & Eapen, 2001). Avoidable yield loss under field conditions was 45.3% due to *M. incognita* but was only 33.3% in a mixed infestation of *M. incognita* and *R. reniformis* (Bai *et al.*, 1995). Pathogenicity studies using *R. similis* showed that an initial inoculum level of ten nematodes per plant can cause a reduction of 35% of the rhizome weight after four months and 46% reduction at the end of the season (8 months). With 100,000 nematodes, the extent of reduction in rhizome weight is 65 and 76%, after 4 and 8 months respectively (Sosamma *et al.*, 1979).

### ***Symptoms of damage***

Turmeric plants infested with

*M. incognita* have stunted growth, yellowing, marginal and tip drying of leaves and reduced tillering with galling and rotting of roots. In the field, high densities of *M. incognita* cause yellowing and severe stunting and withering in large patches. Plants die prematurely leaving a poor crop stand at harvest. Infested rhizomes tend to lose their bright yellow colour (Mani *et al.*, 1987).

Roots of turmeric damaged by *R. similis* become rotted and most of these decayed roots retain only the epidermis devoid of cortex and stelar portions. The infested plants show a tendency to age and dry faster than healthy plants. Infested rhizomes are of a yolk yellow colour compared with the golden yellow colour of healthy rhizomes and have shallow water-soaked brownish areas on the surface. The scale leaves also harbour *R. similis* (Sosamma *et al.*, 1979).

#### **Management measures**

Control has not been studied under field conditions. However, use of clean, nematode-free rhizomes for planting should be the first step in developing an integrated management system for the nematodes of turmeric.

Cultural control: Immersing turmeric rhizomes in hot water at 55°C for 10 min or 45°C for 50 min can kill *M. incognita* inside rhizomes (Chen *et al.*, 1986) and this could be used for establishing nematode-free multiplication plots but is unlikely to be economic for large scale field use.

Host resistance: The cultivars and breeding lines 5379-1-2, 5363-6-3, Kodur, Cheyapuspa 5335-1-7, 5335-27, Ca-17/1, Cli-124/6, Cli-339, Armoor, Duggirala, Guntur-1, Guntur-9, Rajampet, Sugandham and Appalapadu have been reported as resistant to *M. incognita* (Mani *et al.*, 1987;

Gunasekharan *et al.*, 1987). The species *C. zedoaria* is more resistant to *M. incognita* than *C. domestica* in China (Chen *et al.*, 1986). In Andhra Pradesh, India, the high yielding varieties like PCT8, PCT10, Suguna and Sudarshana were free from root knot nematode infestation (Rao *et al.*, 1994). Recently eight turmeric accessions (Acc. Nos. 31, 82, 84, 142, 178, 182, 198 and 200) were identified as resistant to root knot nematode (Eapen *et al.*, 1999).

Chemical control: Application of DBCP at 15 l a.i./ha 15 days prior to planting results in a yield increase of 253-270% compared with 59-187% increase in yield with application of phenamiphos at 2.5 kg a.i./ha one day before planting (Patel *et al.*, 1982). Aldicarb and carbofuran applied at 1 kg a.i./ha increased yield by 71% and 68% respectively over control, with a cost benefit ratio of 1:6 in aldicarb and 1:2 in carbofuran treatments (Gunasekharan *et al.*, 1987). Carbofuran at 4 kg a.i./ha applied in rows to 4-month-old turmeric crop has resulted in a 81.6% reduction in root-knot nematode population as against 45% increase in untreated plots (Mani *et al.*, 1987). Similarly, application of carbofuran or phorate (@ 1 kg a.i./ha) reduced root knot nematodes (Haidar *et al.*, 1998b).

Biological control: Biocontrol agents like *V. chlamydosporium*, *P. lilacinus*, *Fusarium* sp., *Aspergillus* sp. and *Scopuloriopsis* sp. controlled root knot nematodes in field trials (Ramana *et al.*, 2002).

#### **Future prospects**

Turmeric has received very little input in terms of nematological research. Although several nematodes are known to damage the crop, *Meloidogyne* spp. are the most important plant nematodes damaging turmeric in

Kerala and other states. Severe damage due to *Pratylenchus* spp. is reported from Andhra Pradesh but not from other regions. Detailed investigations including surveys, pathogenicity experiments etc. are warranted. Screening for resistance/tolerance to root-knot nematodes has indicated the availability of resistance in several germplasm accessions. This should be profitably exploited to develop resistant cultivars, which can be the best and most economical means of managing them.

### Other Spices

A number of other spice crops like clove, nutmeg, cinnamon, cassia, tamarind, kokum, vanilla etc. are cultivated over large areas in Kerala. However, very little information is available on the damage and yield loss caused by plant parasitic nematodes on many of these crops. In some random surveys, a few nematodes are reported on some of these crops (Ghesquiere, 1921; Goffart, 1953; Goodey *et al.*, 1965; Kumar *et al.*, 1971; Sharma & Loof, 1974; Bridge, 1978; Sundararaju *et al.*, 1979b; Chawla & Samathanam, 1980; Orton Williams, 1980; Dasgupta & Rama, 1987; Rama, 1987; Stier, 1984 in Bridge, 1988).

### Thrust areas

The humid, tropical agro-ecosystem prevalent in spice tracts in association with the high-density multi species cropping system is very congenial for nematodes. Naturally the nematode biodiversity will be at its maximum in such an environment. This warrants an intensive study on the genetic diversity of nematodes affecting various spices. This is more pertinent in view of intensifying our efforts to develop resistant spice plants. Diverse populations of root knot and burrowing nematodes are collected and

maintained at the institute to study their genetic diversity. Intra-specific variations in these populations are assessed through host range studies, morphological and molecular analyses. Molecular tools are also used to develop diagnostics for nematodes. The rich microbial diversity in the tropical soils can be exploited to develop ideal biological weapons for managing plant parasitic nematodes. The institute is focusing on endophytic and rhizobacteria for this. The abundant genetic variability available in spice crops is screened for locating resistance to nematodes. Biotechnological tools are being increasingly used to develop nematode resistant plants.

### Conclusions

In general, spice crops constitute a major group of cash crops that have profound influence on the economy of the state. However, the nematological studies are mostly focused on a few spice crops like black pepper, cardamom, ginger and turmeric. For many of these spices discussed, there is a definite lack of information on some critical aspects. The survey work, which is still incomplete, is not supplemented with crop loss assessment studies. Chemical management of nematodes is ruled out in many spices due to the mass drive towards organic spices. Therefore alternate eco-friendly measures to be developed and their cost-benefit relationship is to be worked out. Studies on nematode damage to these important crops and their economic importance needs to receive greater attention in future.

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## NEMATODES OF TUBER CROPS

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Tuber crops are a heterogenous assemblage of cultivated plants varying in different characteristics and representing different families of the plant kingdom. The crops are identical only in having edible tubers in the underground portion of the plant. They form the third most important group of food crops of man after cereals and grain legumes. The tubers are rich in carbohydrates and thus provide energy rich food. They also vary in size, shape, taste and in their utilities to man and his animals. The different crops also vary in their duration and ability to grow successfully under different hostile environmental conditions such as near drought, poor soils, shades and poor management conditions requiring less labour. The most important tuber crop grown in India and the world over is cassava followed by sweet potato. Other important groups of tuber crops are the yams and aroids. The minor tubers grown in India include the arrow root (*Maranta arundinacea*), yam bean (*Pachyrrhizus erosus* L.), winged bean (*Psophocarpus tetragonolobus*) and chinese potato (*Solenostemon rotundifolius*).

Among the pests of tropical tubers, nematodes are almost a neglected group. They not only reduce tuber yield but also bring down the quality of the tubers. Infested tubers are smaller in size, often malformed with branching and irregular wart like surface protuberances. All of these lower the

marketability of the tubers. Besides, the nematodes harboured in the tubers multiply during transport and also during post-harvest storage and continue to cause further damage.

Though nematodes were reported from various regions of the world on different crops, the importance of the damage to the tuber crops is underestimated in most of the cases. Probably this may be due to the occurrence of high degree of resistance to nematodes in tuber crops (Mohandas and Palaniswami, 1996). As the nematodes feed and multiply on the edible portion of the tubers, the affected tissues look reddish brown while peeling and the taste too deteriorates upon cooking. All these made the farmer recognize that some problems were associated with a particular tuber and prompted him to reject the infested tuber. Besides, as the nematodes continue to multiply even after harvest and during storage, the seed materials which were severely infested were inadvertently selected out during the next planting season. These two types of selections led to the elimination of infested materials which in turn helped in the evolution of resistant cultivars among tuber crops. A large number of resistant tuber crops have already been identified in cassava, sweet potato, yams and aroids. Besides, the harvesting of tuber crops is also different in that the whole plant has to be uprooted while harvesting the tubers. This requires digging of the field which is

almost equivalent to summer ploughing and usually the field is left fallow throughout the next summer and is planted only in the next season. These operations make the field inhospitable for parasitic nematodes and the residual population in soil is often wiped out unlike in other crops where the plant remains intact in the field.

Despite the lack of awareness of the damage done by nematodes in tuber crops, the yield loss in these crops is more than in other cultivated crops. Lot of research efforts are to be undertaken to estimate the actual loss due to nematodes and also for the management of nematode problems associated with different tuber crops.

### 1. Cassava

Cassava is the only species of the genus *Manihot* (*M. esculenta* Crantz) under cultivation. Though a native of Brazil (Smith 1968), North Amazonia has been reported to be the place of its domestication (Nassar, 1978). Globally, about 153.69 million tonnes of cassava is produced from an area of 15.67 million ha. In India, cassava is restricted to the Southern and North-eastern regions, where it is used as food, feed and fodder. It has a number of industrial uses too. Cassava performs well in marginal soil with average or little management and is tolerant to a number of diseases, pests and also near drought conditions.

A number of plant parasitic nematodes have been reported to be associated with cassava though detailed reports on yield loss and management of the nematodes are not available. The root knot nematode is considered to be one of the important nematodes of cassava. Bridge *et al.* (1991) reported that *Meloidogyne incognita* infested cassava in farmers field in Uganda and up to 80 females

of the nematode were recorded from one gram root tissue. Coyne (1994) recorded losses upto 98% in storage root yield under heavy infestation by *Meloidogyne* spp. Caveness (1980) reported that in a glass house experiment cassava seedlings were found to be highly susceptible to *M. incognita*. Other species of *Meloidogyne* reported on cassava from elsewhere are *M. arenaria* and *M. hapla*. Among *Pratylenchus* sp., *P. brachyurus*, and *P. safaenis* have been reported on cassava. Other nematodes reported on cassava are *Rotylenchulus reniformis*, *Helicotylenchus erythrinae*, *H. dihystra* and *Scutellonema bradys* which are of lesser importance to the crop.

*M. incognita* and *M. javanica* are the most important and widespread pest of cassava among all the nematodes in Kerala (Mohandas, 1994). The nematodes produce small individual galls on roots. More galls are found on feeder roots with egg masses protruding outside. More galls are found on young cassava plants less than two months old but as the plant grows fewer galls are noticed indicating juvenile susceptibility and adult resistance. Studies taken up on pathogenicity indicated that 1000 juveniles of *M. incognita* is the threshold level for causing significant damage to susceptible cassava variety H 165. Other nematodes recorded in Kerala are *Pratylenchus* sp., *Hoplolaimus* sp., *Tylenchus* sp., *Tylenchorhynchus* sp. and *Xiphinema* sp. The *Helicotylenchus* spp. associated with cassava were identified as *H. indicus*, *H. multincinctus* and *H. crenacauda*. *R. reniformis* was also found associated with the tuber.

Control of nematodes using nematicides is not desirable in cassava especially because of the residues it may leave behind in the tubers and also in the environment. More-



Table 1: Reaction of indigenous cassava accessions to root-knot nematode, *Meloidogyne incognita*

Germplasm accessions screened	Mean nematode population / g root (range)	Root-knot index
CI 8, 11, 12, 13, 17, 19, 21, 27, 38, 39, 43, 45, 52, 57, 58, 59, 63, 69, 73, 78, 81, 83, 93, 97	0	0
CI 3, 4, 5, 6, 22, 23, 34, 44, 53, 67, 74, 84, 88, 94	160.5 (50-310)	0*
CI 1, 7, 10, 16, 18, 25, 33, 41, 46, 49, 51, 55, 61, 62, 68, 71, 72, 75, 76, 80, 85, 86, 89, 95	434.71 (120-950)	1
CI 20, 32, 42, 47, 48, 50, 60, 65, 66, 70, 82, 87, 90, 92, 93, 96	910.95 (450-1600)	2
CI 14, 24, 26, 35, 40, 64, 77	2070.71 (1820-2300)	3
CI 9, 28, 79	2740 (2600-2820)	4

\* indicate no visible galls

over, as this is a long duration crop more than one application of the chemical will also be required. Use of resistant varieties appears to be most desirable for the management of the root-knot nematode problem. A number of authors reported resistance in cassava cultivars and germplasm accessions to root-knot nematode. Mohandas and Palaniswami (1996) reported resistance in four cultivars of cassava from Kerala. Sreeja *et al.*, (1998) screened ten cultivars / varieties and found that three were resistant to the nematode. Out of 208 germplasm accessions screened, 120 belonged to exotic collections and 88 were indigenous. Majority of the accessions were immune and highly resistant. Only a few accessions were found to be susceptible.

## 2. Sweet potato

South America has been widely recognized as the centre of origin of sweet

potato. Though the genus *Ipomoea* consists of about 175 species, *I. batatas* is the only tuber bearing species even though a few species are known to have thickened roots (Rajendran and Easwari Amma, 1994). Sweet potato is grown in all parts of the tropical and sub-tropical world and in warmer areas of the temperate region. Area under sweet potato during 1991 was 9.26 million ha with a production of 126.18 million tonnes. In India, it is grown in 19 states and two Union territories, but bulk of the area is confined to Orissa, Bihar and U.P. (Chadha and Nayar, 1994).

Among the nematodes of sweet potato, species of *Meloidogyne* and *R. reniformis* cause reduction in yield and also quality of tubers the world over (Clark Moyer, 1988). *Pratylenchus* spp. is reported to be very serious in Japan whereas *Ditylenchus destructor* and *D. dipsaci* are reported to be quite serious in China (Sharma *et al.* 1997).

## 2.1 Root-knot nematode

*M. incognita* is the most important species of *Meloidogyne* attacking sweet potato. Other species include *M. javanica*, *M. hapla* and *M. arenaria*. Infestation by *Meloidogyne* spp. causes reduction in yield and quality. Gapasin (1981) reported that *M. incognita* reduced tuber production from 10.2-47.7 % in Philippines. In North Carolina yield of sweet potato in the nematode infested sandy soils was up to one third compared to nematicide treated plots; cracked roots in infested plots were about 18% compared to 3% in treated plots (Nielsen and Sasser, 1959).

The root-knot nematode infesting sweet potato in Kerala was identified as *M. incognita*. The nematode enters inside the root and also tubers of sweet potato. In root, it produces the typical galls. In tubers, wart like projections are seen on the surface. Significant reduction in shoot weight was recorded in inoculated plants of susceptible accessions/ varieties. Pathogenicity trial of root-knot taken up on a released variety 'Sree Retna' indicated that as initial inoculum level increased there was corresponding reduction in plant growth characteristics. In general, fibrous root weight was low in infested roots compared to the uninoculated control. Increase in root galling in both feeder roots and tubers, population / g root and final soil population were higher in plants inoculated with higher inoculum levels. Splitting of tubers was observed in plants which received higher inoculum. The eggs were laid in gelatinous matrix inside the root and tubers. In the fibrous root, the egg masses were found protruding outside. Other plant parasitic nematodes observed associated with the crop were *R. reniformis*, *H. dihystra*, *Hoplolaimus indicus*, *Tylenchorhynchus* sp., *Tylenchus* sp., and *Xiphinema* sp.

## Chemical Control

Nematicides like nemacur and aldicarb were found to be effective in controlling *Meloidogyne* species (Clark *et al.*, 1980; Gapasin 1981). Control of nematodes in sweet potato using chemicals in California was restricted to preplanting fumigation with methyl bromide (Roberts and Schueurman, 1984). Application of DS 38697 significantly reduced *M. incognita* in *I. batatas* (Averre *et al.*, 1985). Preplant nematicidal treatment of *M. incognita* infested field doubled the yield of marketable sweet potato roots and also reduced proportion of cracked tubers by over 40% (Hall *et al.*, 1988). Kistner *et al.* (1993) reported 11.4% decrease in marketable yield due to infestation by *M. incognita* and *M. javanica* in South Africa.

## Host Resistance

High degree of resistance to *M. incognita* and *M. javanica* has been reported from the world over and high yielding released varieties are available in many countries including India, USA, China and Japan. High yielding varieties namely 'Satsumahikari', 'Excel', 'Yushu 3', 'Fusabeni Topa 3', 'Hi-Starch', 'Red glow' and 'J-red' which show resistance to nematodes had been released in Japan (Kukimura, *et al.*, 1989). A number of germplasm accessions, cultivars/varieties were observed resistant to the root-knot nematode in Peru (Jatala, 1989; Jatala and Bridge, 1990) and also in India (Mohandas and Palaniswami, 1990a; Mohandas and Rajendran, 1993; Ramakrishnan, *et al.*, 1997). Active breeding programmes are being conducted in US, Japan and China (Sun and Chen, 1994).

Sree Bhadra, a high yielding released variety of sweet potato in Kerala was

Table 2: Immune accessions of sweet potato germplasm

Germplasm accessions	Index	Mean nematode population/ g root
S 1, 2, 16, 17, 18, 24, 29, 34, 35, 36, 37, 38, 39, 48, 49, 50, 53, 54, 55, 59, 62, 65, 66, 118, 154, 167, 170, 172, 223, 246, 274, 286, 318, 501, 505, 507, 533, 553, 568, 571, 582, 587, 588, 594, 600, 601, 606, 610, 611, 612, 622, 623, 624, 625, 628, 629	0	0

Table 3: Resistant accessions of sweet potato germplasm

Germplasm accessions	Index	Mean nematode population/ g root (range)
S 21, 43, 44, 56, 57, 58, 72, 75, 87, 149, 150, 162, 164, 238, 244, 283, 284, 456, 508, 535, 567, 592, 598, 605, 608, 617, 630, 634	0	160.56 (19-400)
S 12, 13, 14, 22, 23, 25, 27, 31, 32, 33, 41, 71, 78, 81, 83, 117, 152, 155, 175, 207, 214, 217, 241, 292, 315, 380, 500, 503, 510, 515, 523, 529, 537, 557, 560, 580, 584, 591, 595, 609, 615, 618, 632, 637	1	324.26 (50-800)
S 4, 6, 8, 9, 22, 26, 28, 46, 61, 68, 69, 70, 80, 88, 156, 204, 208, 215, 222, 227, 256, 288, 312, 313, 316, 406, 493, 506, 525, 526, 527, 559, 562, 565, 572, 581, 590, 593, 603, 607, 620, 635, 641, 642	2	595.29 (110-1620)

identified as a resistant trap crop to root knot nematode. Inoculation studies on rooted cuttings of Sree Bhadra recorded high degree of penetration by the nematode which is comparable to susceptible sweet potato accessions such as 108-2 and 56-1. However, development of the infested juveniles into fourth stage males and adult females could not be recorded indicating that the nematode

was not capable of completing its life cycle in Sree Bhadra. When this variety was planted in a root-knot nematode infested field, the nematode population declined to below detectable level over a single cropping duration of 90-95 days. Subsequent susceptible plants viz coleus and African yam also escaped nematode damage when planted in such fields. Different centres of AICRP on

Nematodes viz KAU, Vellayani; JNKVV, Jabalpur; TNAU, Coimbatore confirmed that root knot nematode population declined in heavily infested fields when Sree Bhadra was planted. However, GAU, Anand, isolate of root-knot nematodes was found to infest Sree Bhadra variety. Sweet Potato varieties such as Khajangod, Sree Vardhini and Sree Nandhini were found to be resistant to root-knot nematode.

Though many released varieties and major germplasm accessions are either immune or resistant to the root knot nematode, a few varieties are susceptible. In such susceptible varieties reduction in shoot and root weight were significant even at a low initial inoculum of 20J/pot.

Germplasm accessions (200) were screened against *M.incognita*. Fifty six accessions were found to be immune as neither galls nor nematode numbers were recorded from the roots or tubers of these accessions. One hundred and sixteen accessions recorded very low populations in root. Another 88 accessions recorded a few galls on roots and supported only low population which were categorized as resistant. Twenty accessions recorded 11-30 galls per root. These were recorded as the susceptible accessions.

### 2.2 *Rotylenchulus reniformis*

Gapasin and Valdez (1979) observed 60.6 per cent reduction in yield due to *R.reniformis* pot culture experiments. Gapasin (1981) reported that *R. reniformis* reduced the yield from 13.4-60.6 per cent in Philippines. Walters and Barker (1994) studied the effect of two populations on sweet potato and found that both populations restricted storage root growth but enhanced shoot growth. Besides, infestation by the

nematode may cause cracking of storage roots (Clark and Wright, 1983).

Community analysis of plant parasitic nematodes prevailing around rhizosphere region of sweet potato in Kerala revealed that *R. reniformis* recorded cent per cent absolute frequency. This nematode recorded the highest absolute density and prominence value. Hence more studies on its pathogenicity and yield loss in sweet potato is to be taken up.

### 2.3 *Pratylenchus* sp.

Rajendran *et al.* (1972) reported high population of *Pratylenchus* sp. and *Hoplolaimus* sp. in soil and roots from Coimbatore, Tamil Nadu. *P.coffeae* was very serious in Japan in volcanic acid soils (Suzuki, 1989). Other species reported feeding on sweet potato were *P. flakkeniss*, *P. brachyurus*, *P. penetrans*, *P. vulnus* and *P. zae*.

### 2.4 *Ditylenchus* sp.

In China the nematode is a serious problem and programmes on management and breeding for resistance are in full swing. *Ditylenchus* spp (*D. destructor*, *D. dipsaci*) induce "brown ring" in storage root. A number of resistant accessions and varieties were reported from China (Wu and Zhang, 1990; Wang *et al.*, 1995) Lin *et al.*, 1993 observed that the exudate secreted by the oesophageal glands of *D. destructor* when inoculated on tubers of susceptible sweet potato induced browning of the tissues around the inoculated area and destroyed the cells. In resistant cultivars, the walls turned brown and parenchyma cells became cork barriers.

*Ditylenchus* spp. which are observed to be very serious in China and Japan have not been reported from sweet potato in India. Hence steps should be taken to prevent the

entry of such nematodes through planting materials, when imported.

The burrowing nematode, *Radopholus similis* has been reported to be a serious pest of sweet potato (Koshy and Jasy, 1990).

### 3. Yams

Yams are cultivated in an area of 2.5 million ha with a production of 23.89 million tonnes in the world. Two species of yams are mainly grown in India. They are the lesser yam (*Dioscorea esculenta*) and the greater yam (*D. alata*). The African white yam (*D. rotundata*) was recently introduced in India from Africa (Chadha and Nayar, 1994). Another yam grown in different parts of the world especially China is the yellow yam (*D. cayenensis*). Species of *Dioscorea* are also grown for extraction of diosgenin.

The three important nematode pests of yams are root-knot nematode, yam nematode and lesion nematode. Root-knot nematode infestation produces deformed tubers with uneven surface, whereas the other two nematodes produce typical 'dry rot' of tubers. *Rotylenchus blaberus* was reported to cause a disease in yams in Nigeria (Steiner, 1937).

#### 3.1 Root-knot nematode

The root-knot nematode *M. incognita* is the major nematode infesting yams the world over. In China *M. arenaria* were reported to be very serious on Chinese yam (Gao *et al.*, 1992). *D. esculenta*, *D. alata* and *D. rotundata* are found to be highly susceptible to the nematode in Kerala. Infestation by the nematode leads to wart like projections on tuber surface and reduction in size of tubers in *D. alata*. In *D. esculenta*, severe infestation resulted in the production of small sized tubers in bunches which were unfit for consumption. In *D. rotundata* profuse roots were recorded on infested tubers at harvest.

Reduction in yield was reported by many authors from different countries and the most serious infestation may lead to rapid senescence and early death of young plants. Mohandas and Ramakrishnan (1997) reported the pathogenic effect of *M. incognita* on the African white yam. Fresh and dry weight of roots and tubers were significantly reduced in treatments receiving 100 or more nematodes per plant. Infestation resulted in browning of tuber tissue and subsequently rotting. Delay and reduction in germination of infested tubers were also observed in *D. rotundata* and *D. cayenensis* (Hutton *et al.*, 1978). The nematode also grows and multiplies during storage, on *D. alata* and *D. rotundata* causing further damage to the tuber (CTCRI, 1997). Apparently healthy and less infested tubers developed severe galling during storage, whereas heavily infested tubers dried up (CTCRI, 1995). Moisture loss during storage is reported to be over thirty per cent whereas it is only around ten per cent in healthy tubers (Cadet and Quencherve, 1994). Oxygen uptake by *D. rotundata* and *D. cayenensis* was significantly higher in root-knot nematode infested tissues.

As is the case with cassava and sweet potato, resistance is very common in yams in India. Rajendran and Sivagamivadivelu (1990) screened cultivars and reported no resistance on both *D. alata* and *D. esculenta* from Coimbatore. Very high degree of resistance was identified in *D. esculenta* and *D. alata* (CTCRI, 1996). High yielding and released varieties from CTCRI, viz. 'Sree Latha' of *D. esculenta* and 'Sree Keerthi' of *D. alata* were found to be highly resistant to the root-knot nematode. Out of 33 *D. alata* accessions tested against *M. incognita*, 3 were immune (Da 66,7 120, 228),

Da 199 was highly resistant and 9 were resistant (Mohandas *et al.*, 1996).

Though chemical treatments for control of these nematodes are effective, this may not be practical because of the long duration of the crop. Selection of healthy seed materials will be ideal. Hot water treatment of setts used for planting is found to be effective in checking the nematode. Soaking the setts in 1200 to 2400 ppm of oxamyl for 40 minutes was most effective (Hutton *et al.*, 1978 and Roman *et al.*, 1984). Carbofuran, fen sulphothion or aldicarb were also effective in controlling nematodes (Roman *et al.*, 1984).

Resistant varieties may be planted in infested fields. There was no damage to susceptible *D. rotundata* when it was preceded by resistant Sree Bhadra variety of sweet potato (CTCRI, 1997). Ayala and Acosta (1971) also reported reduction of nematode fauna following rotation with *Zea mays*, *Capsicum annum* and *Nicotiana tabacum*.

### 3.2 The yam nematode

*Scutellonema bradys* is one of the most important pest infesting yams. This nematode was recorded on yams from India, West Africa, the Caribbean and Brazil (Bridge, 1982). The nematode produces 'dry rot' on yams (West, 1934). In field, the damage is restricted to the outer thin layer of the tuber (Adesiyon, 1977). In a survey of nearly 500 samples collected from French West Indies, all *Dioscorea* cultivars were found infected by *S. bradys*. In the tubers, *S. bradys* may be found alone or associated with *P. coffeae* (Castagnone-Sereno, *et al.*, 1988). However, in store the nematode infection spreads and infection sites coalesce which encircle the tuber and could result in 80 to 100 per cent loss of stored yams (Adesiyon and Odihirin, 1975). Cadet and Quencherive (1994) also

reported serious damage of yams during storage and the weight loss reached upto 30%, whereas in healthy tubers it was only 10%.

Immersion of tubers for 40 minutes in water at 50°C or oxamyl was recommended before planting for the control of *P. coffeae* and *S. bradys* (Castagnone-Sereno, 1988). Coating of yam seed tubers with liquid ethoprophos and cadusafos also controlled root population of *S. bradys* but did not produce significant increase in yield (Cadet and Daly, 1995).

### 3.3 The lesion nematode

*P. coffeae* was recorded from Jamaica, Puerto Rico and British Solomon Islands (Bridge, 1982). The symptoms are very similar to that of 'dry rot' caused by *S. bradys*. This was also recorded from India on *D. alata* and *D. rotundata* (CTCRI, 1994). In store the nematode continue to multiply and damage the tuber surface. Under severe infestation the rotting coalesces and encircles the entire tuber.

## 4. Aroids

Information on area and productivity of aroids is scanty as their cultivation is scattered mostly in homesteads. In India *Colocasia esculenta* and *Xanthosoma sagittifolium* are grown predominantly and are used as vegetables in various parts of the country. The elephant foot yam, is another important aroid grown in different parts of the country which is also used as a vegetable.

Among the edible aroids, the most important are taro (*C. esculenta*), tannia (*X. sagittifolium*) and elephant foot yam (*A. paeoniifolius*) (Pillai, 1984).

### 4.1 *Colocasia* and *Xanthosome*

*M. incognita* and *M. javanica* were

reported on *Colocasia* and *Xanthosoma* from Southern Florida (McSorley, 1980). Damage on these two crops depended mostly upon the cultivar, growing conditions, nematode population and geographic location (McSorley *et al.* 1983b). Crozzoli *et al.* (1995) outlined briefly the damage due to *M. javanica* on *X. sagittifolium* from Venezuela. Runkulatile and Teri (1990) recorded *M. arenaria* and *M. javanica* as dominant species from Morogoro region of Tanzania. Spies *et al.* (1995) evaluated 59 cultivars of colocasia and reported that all were susceptible to *M. javanica* though two cultivars namely Mana Ulaulu and Piko Ulaulu recorded low levels of nematode reproduction and less damage suggesting that these cultivars are source of "partial resistance and tolerance" to *M. javanica*. Sides and Arakaki (1971) outlined the importance of cover crops in reducing damage by *M. incognita* to *C. esculenta*, in a tropical cropping system.

Nirula (1959) reported root-knot nematode on colocasia in India. Nadakal and Thomas (1964) reported the nematode on colocasia from Kerala. Srivastava *et al.* (1969) reported damage caused by *M. javanica* and discussed its control. Mohandas and Palaniswami (1990b) reported reduction in yield upto 21.4 per cent in colocasia var. Sree Pallavi. Infected cormels were deformed and smaller in size with branches which affected the marketability of the tubers. Roots recorded typical galls. Resistance also was reported by them in Sree Reshmi, a high yielding released variety and C9, a popular cultivar. In Kerala simultaneous infestation of root-knot and burrowing nematodes were reported on *Xanthosoma*.

#### 4.2 *Amorphophallus*

Mohandas (1994) reported damage due

to infestation by the root-knot and root lesion nematodes. Infestation by the former produce typical galls on the roots. In corms and cormels infestation leads to irregular projections, which are relatively more on the latter. Under severe infestation, the tuber tissue gets discoloured and in corms the infested area dries up, a situation resembling dry rot of yams. The nematode was observed from Andhra Pradesh, Kerala and Tamil Nadu by the author. Farmers in Kerala keep the pest under control by planting nematode free healthy corms. They also practice crop rotation. In Andhra Pradesh the nematode is a very serious problem and severe infestation lead to rotting of the tuber before harvest. The root knot nematode multiplies in the tuber after harvest during transport and in store, causing more damage. Premature sprouting was also recorded, when the infestation was severe. Infestation by lesion nematode change tuber colour to black with cracks on the surface. Infestations by both the nematodes were found to be very high in irrigated fields as compared to rain fed tracts.

No resistance has so far been reported in *amorphophallus* to root-knot nematode. Farmers in Kerala practice crop rotation, planting healthy nematode free corms and rotation of the crop with non host crops are the only way to keep the crop free from infestation. The root-knot nematode population present in the soil multiply and build up in the first year and normally they infest roots and the cormels and rarely corms. Hence corms escape nematode damage in the first year. But when the crop is taken up continuously the population build up is so high that they enter into corms too and cause severe damage. Application of neem cake at the time of planting in pits was found to be effective for the management of the nematode.

Sheela *et al.*, 2004 reported the effect of *Bacillus macerans* seed material treatment (0.3 per cent w/w of seed material) for the management of root-knot nematode. The incremental yield due to the above treatment was 41,500kg/ha.

Other nematodes infesting amorphophallus are *R. similis* and *Radopholoides* sp. Infestation by *R. similis* caused rooting of tuber tissue. The infested roots were devoid of cortical layer. However, infestation is rare.

## 5. Coleus

Coleus (Koorka) *Solenostemon rotundifolius* (Poir) Morton is a short duration under exploited tuber yielding vegetable mainly cultivated in Kerala as well as Tamil Nadu. In Kerala this crop was extensively grown in northern districts. Of late, the demand for tubers has fuelled the cultivation in the southern districts of Kerala also. The coleus tubers are rich in carbohydrates (18-21 per cent), minerals like calcium and iron, vitamins like thiamine, riboflavin, niacin and ascorbic acid. One of the major constraints in the production of tubers is the losses sustained due to attack by insects, diseases and nematodes.

### 5.1 Root-knot nematode : *Meloidogyne incognita*

Coleus is highly susceptible to this nematode. Sathyarajan *et al.*, (1966) reported root-knot nematode, *Meloidogyne incognita* infestation in *S. rotundifolius* from Kerala. Patnaik and Das (1986) studied the pathogenicity of *M. incognita* on coleus. Due to the attack of *M. incognita* in *S. rotundifolius* conspicuous gall like swellings are found in the roots, resulting in the malformation of tubers, unfit for immediate consumption as well as for storage. Coleus being a tuber crop,

attacked by soil inhabiting nematodes occur in the roots and tubers in the soil. The nematodes continue to multiply inside the tuber after harvesting during transportation and storage. Mohandas *et al.*, (1988) in a pathogenicity experiment reported that dry weight of tuber was reduced to 5 per cent compared to 20 per cent in the control. The nematode was seen continue to feed and multiply during storage. More pronounced galls appeared in mildly infested tubers under storage whereas heavily infested ones rotted (Mohandas and Ramakrishnan, 1998). The quantity and quality of the produce also get deteriorated. The per cent of starch on fresh weight was 16 in control and 2 under the highest inoculum (CTCRI, 1988)

### Yield Loss

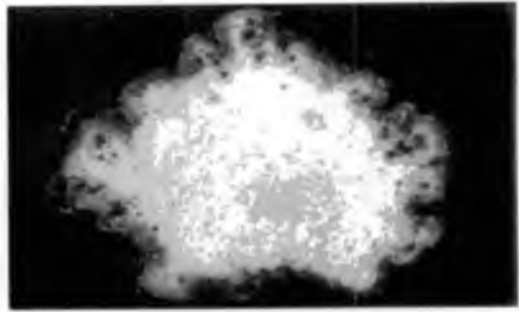
The crop loss due to *M. incognita* was 92 per cent (in tune of fresh weight of tuber) at an inoculum level of 10,000 *M. incognita* larvae per plot (Sosamma, 1988). The extent of loss due to *M. incognita* was estimated by Nisha (2005) at an initial inoculum level of 100 juveniles onwards. At 100 to 5000 J<sub>2</sub> plant showed 14-16.38 per cent in number of branches respectively. The weight of total marketable and edible portion of tubers, reduction being 19.43-45.03, 16.05-44.75 and 19.61-49.84 per cent respectively at 100 and 5000J<sub>2</sub> levels. (Figure). This will give a prediction model for planning management strategy.

Crop loss studies under storage condition revealed significant reduction in weight, germination and vigour of plants raised from tubers collected 100J<sub>2</sub> inoculum levels. The weight loss in tubers was 12.5, 86.8 and 96.85 per cent in uninoculated, 100J<sub>2</sub> and 500J<sub>2</sub> respectively. The tuber collected from uninoculated plants showed cent per cent germination while in others





Root-knot infested *Dioscorea alata*



C.S. of *D. alata* female and eggmass on the peripheri



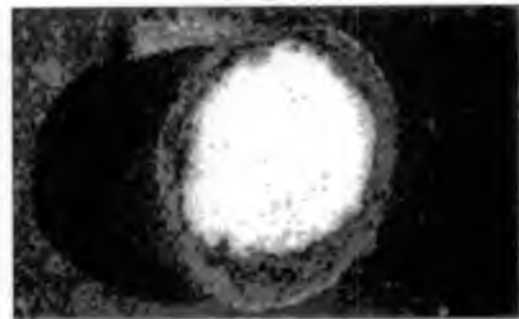
Root-knot infested *D. esculenta*



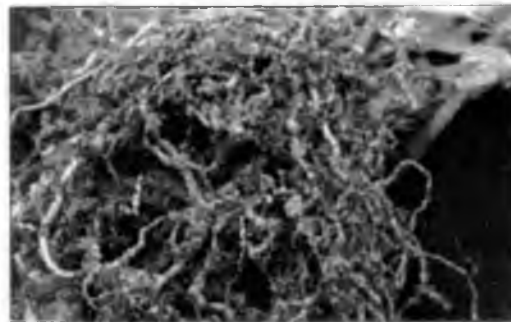
Root-knot infested *D. rotundata*



Yams with dry rot by *Scutellonema bradys*



*D. rotundata* infested with *Pratylenchus*

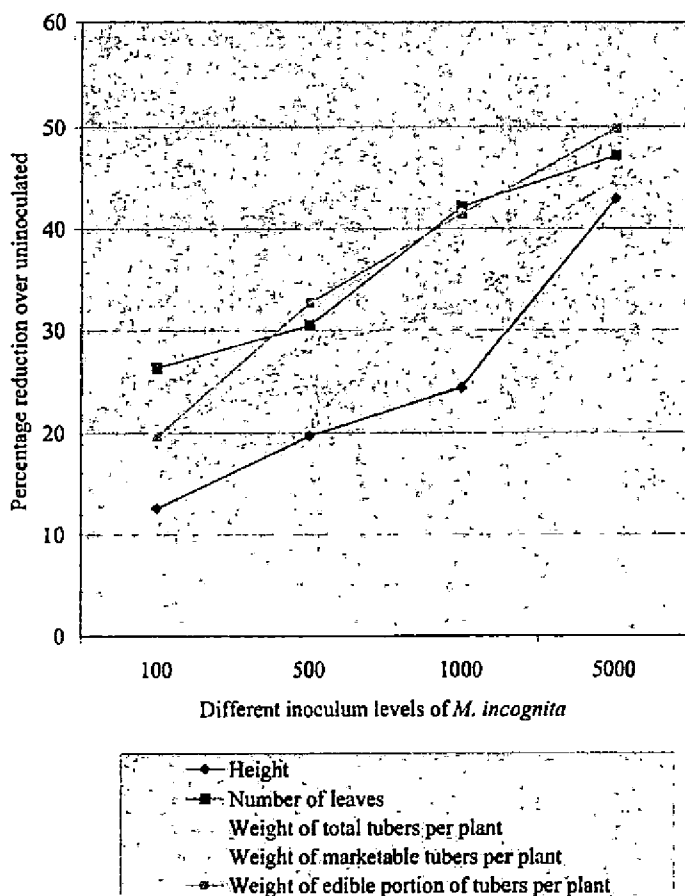


Root-knot infested coleus root



Root-knot infested coleus tuber





Effect of different inoculum levels of *M. incognita* on the biometric characters and yield of *S. rotundifolius* at the time of harvest

it was only 42.37 and 9.47 respectively. (Nisha, 2005).

The biochemical changes in tubers of *S. rotundifolius* infested by various levels of *M. incognita* were assessed in terms of protein, starch, sugar and crude fibre contents. The reduction being 6.32 to 33.33, 8.06 to 17.47 and 18.99 to 62.03 per cent respectively as the population of the nematodes increased from 100 to 5000  $J_2$  level of *M. incognita*. However the protein content showed an increase of 12.94 to 14.42 per cent over uninfested coleus tubers (Nisha, 2005).

### Management

Development of the concept of pest management and the implementation have led to a greater appreciation of the need for a wide range of tactics for nematode control. Sole reliance on pesticide is not sustainable because of the associated problems of environmental degradation and secondary outbreak due to climate of natural enemies. Other effective and environment friendly methods have to be adopted and this trend has gained momentum in recent years. One among them is the use of microbial pesti-

cides. When used as a part of IPM programmes, microbial pesticides (bacteria, fungi, nematodes, protozoan and viruses) are viable, safe and eco-friendly alternatives. Since coleus is a short duration crop and tubers are the consumable portion, the application of chemical pesticides in soil will adversely affect soil microflora and fauna resulting in very high level of pesticide residue in harvested produce. By developing sustainable biointensive integrated management strategy using combination of techniques and practices giving emphasis to biocontrol agents and other non-chemical measures, the *M. incognita* population can be maintained below the threshold levels and the over dependence on pesticides can be avoided.

The resistant screening of the varieties / lines of coleus against *M. incognita* revealed that the var. Sree Dhara and Nithi were showing resistance and recorded the minimum root-knot index of 1.00.(Nisha, 2005).

Sheela *et al.*, 2004 reported the beneficial effect of drenching of the bioinoculant *Bacillus macerans* (2 per cent solution) for the management of nematodes associated with coleus. The economic analysis of coleus yield revealed that *B. macerans* drenching alone gave an additional yield of 10,675kg/ha in comparison to untreated control. The standard biocontrol agent *Paecilomyces lilacinus* drenching, the increase in yield was only 4550kg/ha.

For formulating an integrated nematode management strategy initial screening of various treatments from nursery to main field were studied. Integration of solarization of nursery and application of *P. lilacinus* along with either neem cake or *B. macerans* in the main field and nursery application of *P. lilacinus* in combination with *P. lilacinus*+

neem cake in the main field were superior to carbosulfan in improving the height, plant spread and leaf area. Combination of solarization of nursery and application of *P. lilacinus* together with *B. macerans* in the main field exhibited significant superiority over the untreated in improving the weight of marketable tubers per plant. Regarding the weight of edible portion of tubers the treatment combination of nursery solarization or application of either *P. lilacinus* or *B. macerans* in the nursery along with application of *P. lilacinus* in combination with neem cake or *B. macerans* in the main field significantly reduced *M. incognita* population in soil and root.

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# NEMATODES OF MEDICINAL AND AROMATIC PLANTS

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Medicinal and aromatic plants are high valued remunerative crops being used as raw materials for flavouring, pharmaceutical, perfumery and cosmetic industries of the world. In olden days, their availability was through collections by men from mountains and inaccessible areas and were considered as rare commodities. With the advancement in agriculture and increase in demand, cultivation of medicinal and aromatic plants started in different parts of Asia, Africa, Europe and United States. Mints, lemongrass, palmarosa, citronella, kacholam, senna, koduveli, davana and basil accounted for the major share in terms of production and usage. Several other plants like geranium, patcholi, belladonna, yams, sarpagandhi, mulathi, ammi, rosemary etc. are restricted in acreage and region, because of their specificity in climatic and geographical requirements.

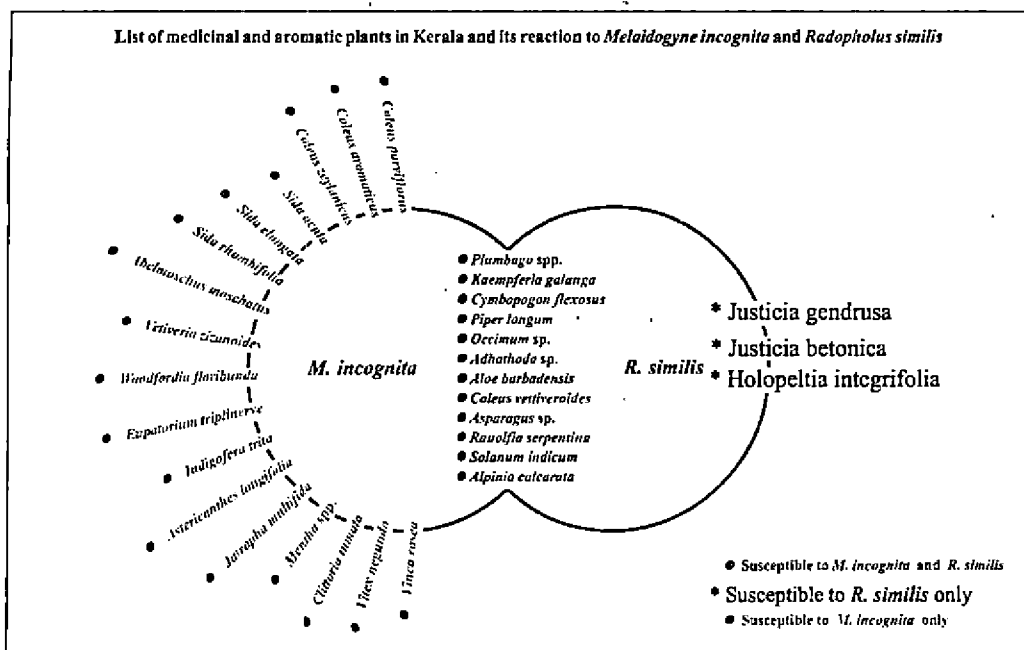
Medicinal and aromatic plants have always found a place in the Indian tradition and culture. Ayurveda, the Indian System of Medicine, is solely based on herbs. Nowadays, essential oil yielding aromatic plants find special use in cosmetics and aroma therapy. In India, the cultivation of these plants is mainly confined to Uttar Pradesh, Haryana, Punjab, Jammu and Kashmir, Madhya Pradesh, Karnataka, Tamil Nadu and Kerala. Though an array of plant species are grown in Kerala, the large scale domestication and product diversification is restricted to plants such as *Plumbago rosea*,

*Kaempferia galanga*, *Piper longum*, *Holoptelia integrifolia*, *Vetiveria zizanoides*, *Cympopogon flexosus*, *Adathoda* spp., *Coleus aromaticus*, *Alpinia calcarata*, *Sida acuta*, *Indigofera trita*, *Vitex negundo* etc.

Plant parasitic nematodes are a threat to the cultivation of medicinal and aromatic plants. In most cases, the tubers are economically and medicinally important, and therefore the feeding by nematodes on these underground plant parts directly affect the quality and quantity of the produce. Nematodes like *Meloidogyne* spp., *Tylenchorhynchus vulgaris*, *Longidorus* sp., *Pratylenchus thornei*, *Hoplolaimus* sp., *Tylenchus* sp., *Helicotylenchus* sp., *Hirschmaniella* sp., *Heterodera* sp., *Xiphinema* sp., *Trichodorus* sp. and *Radopholus similis* have been reported from the rhizosphere of different medicinal and aromatic plants. Most of the information available with regard to nematodes on medicinal plants relates to mint species and to some extent on *Ocimum* spp., *Hyposcyamus* spp., *Artemisia* spp., *Dioscorea* spp., and a few aromatic grasses (Singh, 1997).

## Status of nematological research on medicinal plants in Kerala

Extensive surveys were carried out in Kerala for studying the nematode problems in medicinal and aromatic plants. Forty-two plants were recorded as susceptible to root-knot nematode, *Meloidogyne incognita*



(Sheela, 1994). The susceptible plants included *P. rosea*, *K. galanga*, *P. longum*, *Coleus parviflorus*, *Coleus aromaticus*, *Coleus zeylanicus*, *Sida acuta*, *Sida elongata*, *Sida rhombifolia*, *Abelmoschus moschatus*, *Vetiveria zizanioides*, *Woodfordia floribunda*, *Eupatorium triplinerve*, *Indigofera trita*, *Aesticranthes longifolia*, *Jatropha multifida*, *Clitoria turnata*, *V. negundo* and *Vinca rosea* (Fig.). *Emilia sonchifolia*, an annual herb was recorded as a new host of *M. incognita* (Nisha and Sheela, 2002). The root knot nematode was also reported to induce root rot and ultimately reduce the quantity and quality of the green leaves on long coriander (*Eryngium foetidum* L.) (Sheela et al, 2003).

The plants found susceptible to *R. similis* included *P. rosea*, *K. galanga*, *P. longum*, *Justicia gendrusa*, *J. betonica* and *Holoptelia integrifolia*. Plant species viz. *Cymbopogon flexosus*, *P. longum*, *Ocimum* spp., *Adathoda*

spp., *Aloe barbadensis*, *Coleus vetiveroides*, *Asparagus* spp., *Rawolfia serpentina*, *Solanum indicum* and *Alpinia calcarata* were susceptible to both the nematodes. Since *P. rosea*, *K. galanga* and *P. longum* are cultivated on a large scale for catering to industrial needs, detailed studies were conducted on nematode infestation in the crops.

#### ***Plumbago rosea* (Chethikoduveli)**

Survey conducted in the herbal gardens of Kerala revealed the association of *Meloidogyne incognita*, *R. similis*, *Rotylenchulus reniformis*, *Tylenchorynchus* sp., *Helicotylenchus multicinctus*, *Belanolaimus* sp., *Hoplolaimus* sp., *Criconeoides* sp. and *Hemicriconeoides* sp. in *P. rosea* (Kumar et al., 2001). *M. incognita* and *R. similis* were the major nematodes damaging the crop.

Crop loss assessment studies conducted indicated that *M. incognita* population at different levels (100, 1000, 10000) adversely



affected the biometric characters and yield of *P. rosea*. At the pathogenic level (100 J<sub>2</sub>), the percentage reduction in plant height, number of leaves, branches, fresh weight of root, dry weight of shoot and weight of tubers were 14, 26, 20, 18, 28 and 36 per cent over control (KAU, 2004). *R. similis* caused significant reduction in fresh and dry weight of shoot and root from 10 J<sub>2</sub> onwards in *P. rosea*. Regarding the plant height, number of leaves, branches and leaf area significant reduction was recorded at 100 J<sub>2</sub> level. Significant reduction in yield was also recorded at 100 J<sub>2</sub> level.

Histopathological studies conducted revealed that second stage juveniles of *M. incognita* penetrated the root and established its feeding site in the vascular region including endodermis, pericycle, xylem and phloem cells which underwent hypertrophy and hyperplasia after 10 to 15 days of inoculation, leading to the formation of giant cells and root-knots (Sheela, 2004). *R. similis* entered the roots and started feeding on the cortical region that led to the development of longitudinal burrows underneath the cortical cell layer resulting in necrosis and finally lesions.

Biochemical changes by various levels of *M. incognita* and *R. similis* showed considerable variation in the phenol and total free amino acids. The phenol production was increased with initial inoculum levels. The percentage increase in the total free amino acids at different inoculum levels (10, 100, 1000 and 10000) were 12, 44, 96 and 216 respectively for *M. incognita* and 8, 24, 104 and 240 respectively for *R. similis*. The rate of reduction of plumbagin was less in *R. similis* infested plants.

Management trials were conducted in nematode infested microplots using *Glomus*

*fasciculatum* @ 10g/m<sup>2</sup> with 10<sup>6</sup> spores/g, neem and groundnut cake @ 0.5kg/m<sup>2</sup>, carbosulfan 1kg ai/ha, wood ash 5kg/m<sup>2</sup>, *Paecilomyces lilacinus* and *P. fluorescens* 200 spores/g @ 10g/m<sup>2</sup> with 10<sup>6</sup> spores. Among the various treatments, *G. fasciculatum* effectively controlled the nematodes and produced maximum yield. This was closely followed by *P. fluorescens*. Root-knot count was minimum in carbosulfan treatment followed by neem cake and *G. fasciculatum*. The number of larvae emerged per egg mass was lowest in carbosulfan treatment followed by *G. fasciculatum* and *P. fluorescens*.

The field experiment on management of *R. similis* in *P. rosea* revealed that *G. fasciculatum* @ 10g/m<sup>2</sup> was found to be the best treatment followed by *P. fluorescens* @ 10g/m<sup>2</sup>. The effect of neem cake and ground nut cake @ 100g/m<sup>2</sup> and the green leaves of *Glyricidia maculata* and *C. infortunatum* @ 5kg/m<sup>2</sup> were also found effective in managing the nematodes and improving the biometric characters and the yield of the plant but the treatments were inferior to the above bioagents. (KAU, 2004).

### *Piper longum* (Thippali)

The root knot nematode was found pathogenic to *P. longum* at an initial inoculum level of 10 nematode /plant while *R. similis* was pathogenic at 100 nematode /plant onwards. Crop loss in terms of reduction in plant height (31 per cent) leaf area (9 per cent), fresh weight of root (15 per cent) and fresh weight of shoot (26 per cent) were observed at 100 J<sub>2</sub> level for *M. incognita*. Significant reduction in yield (31 per cent) was obtained when the plants were inoculated with 1000 second stage juveniles.

The burrowing nematode reduced the number of branches, leaf area, fresh and dry weight of root and shoot at level 100 with percentage reduction being 14,5,15,8,23 and 26, respectively. The reduction in fruit weight was expressed from level 100 onwards by the attack of nematodes.

Biochemical studies revealed that there was a significant increase in the production of phenols and total free amino acids with increasing levels of nematodes. Maximum phenol content (0.37mg/g) was observed in *M.incognita* infested roots. The total free amino acid (0.66µg eq.of leucine) was maximum in roots infested with *R.similis*.

Bioagents like *G. fasciculatum* (20g/m<sup>2</sup> having 100 chlamydo spores/g), *P. fluorescens* (10g/m<sup>2</sup> with 10<sup>6</sup> cells/g) and *P. lilacinus* (910g/m<sup>2</sup> with 200 spores/g) and carbosulfan @ 1kg ai./ha promoted the growth and yield of thippali and were equally effective in improving the biometric characters viz. plant height, number of branches, leaf area, fresh and dry weight of shoot and yield in terms of number and weight of fruits. Maximum yield in terms of number and weight of fruits ie.30 and 22 g/plant , respectively was obtained in case of *G. fasciculatum*. Bioagents and carbosulfan suppressed the nematode population in soil to the tune of the 87 to 92 per cent. Carbosulfan and *G. fasciculatum* were effective in reducing the number of galls.

Management trials conducted on *R.similis* showed that *G. fasciculatum*, *P. fluorescens* and carbosulfan improved the biometric characters like plant height, number of branches, leaf area, fresh and dry weight of shoot and yield in terms of number and weight of fruits.

#### ***Kaempferia galanga* ( Kacholam)**

Studies on the biology of root-knot

nematode in *K. galanga* revealed that the nematode completed its life cycle in 37 days and the mean number of eggs observed per eggmass was 130. The hatching of eggs and survival of larvae when tested at different moisture levels (field capacity, permanent wilting point and flooding) revealed that the field capacity was the most suitable. The moisture levels at field permanent wilting point and flooding were found to reduce larval hatching.

*In vitro* studies on the effect of soil pH on hatching of eggs and survival of larvae indicated no significant variation at different levels ranging from 5.0 to 7.5.

The effect of different soil types (forest (Alfisol), red (Oxisols) and laterite (Alfisol),sandy (Entisol) ) on the pathogenicity of root knot nematode of Kacholam was studied. Among these, sandy soil was found to be the best for effective multiplication of root-knot nematode (Rajani, 1998).

The crop loss assessment studies were conducted with inoculum levels of 0,200,500 and 1000 J<sub>2</sub> of *M. incognita* per pot. Results revealed that different levels of *M. incognita* population (200 to 1000 J<sub>2</sub>) had significant adverse effect on the biometric characters and yield. At 1000J<sub>2</sub> level, the percentage reduction in the number of leaves, length of rhizome, weight of root, and weight of rhizome was 43.33, 23.7, 46.3, 63.7 and 43.5 respectively.

Pathogenic effect of *R. similis* on Kacholam was observed from 100 J<sub>2</sub> level onwards. Significant reduction was observed in biometric characters and yield.

Histopathological studies conducted in Kacholam revealed that the entry of second stage juveniles of *M. incognita* occurred within 5 days, which further penetrated the

root tissue to establish feeding site on the vascular parenchyma. Hypertrophy and hyperplasia resulted in 10 to 15 days. The phenol production was also increased due to the nematode infestation. The percentage increase ranged from 27 to 41 (10J2 to 10,000J2). Maximum content of 0.385mg/g was revealed in plants inoculated with 10,000J2. The percentage increase in total amino acids ranged from 23 to 61, between 10J2 to 1000J2 levels.

Micro plot experiments were conducted to study the efficacy of various bioagents, organic amendments, botanicals and chemical treatments on the management of nematodes in Kacholam. The effect of rhizome treatment with bioagents (*G. fasciculatum*, *P. fluorescens*, *Trichoderma viride* 3% w/w) botanicals (neem oil 2%, neem leaf extract 5%, neem oil + garlic (1:1) 2% , neem leaf extract 4%+garlic 1%, nimbecidine 0.2%), hot water treatment 55°C and chemicals (dimethoate 0.1%) revealed that maximum leaf production, shoot and root weight and fresh plant weight were recorded in plants treated with *P. fluorescens* and arbuscular mycorrhizal fungi which were equally effective at 3 per cent w/w.

When the main field was treated with organic amendments like neem cake 200g/m<sup>2</sup>, coir pith 500g/m<sup>2</sup> and saw dust 500g/m<sup>2</sup>, neem leaf 750g/m<sup>2</sup> and bioagents like AMF 300g inoculum/m<sup>2</sup>, *P. fluorescens* 10g/m<sup>2</sup> and *T. viride* 10g/m<sup>2</sup> and carbofuran @ 1kg ai./ha for nematode management, treatments like neem cake, coir pith, sawdust, AMF and *P. fluorescens* were found equally effective in suppressing nematode infestation increasing the yield (Nisha, 2001).

Excepting *Hyptis*, mulching with green leaves of neem, glyricidia, mangium,

clerodendron, calotropis, chromolaena and banana significantly reduced the population of nematodes (KAU, 2004).

### Nematodes of aromatic plants

The plant parasitic nematodes reported from the rhizosphere of citronella, lemon grass, palmarosa and vetiver include *Tylenchorhynchus* spp., *Hoplolaimus* spp., *R. reniformis*, *Pratylenchus* spp., *Heterodera* spp., and *Meloidogyne* spp. Among these, the most common and predominant nematodes reported are *Tylenchorhynchus* and *Pratylenchus* (Pandey, 1994). Pathogenicity studies indicated that these nematodes reduced the growth of plant as well as yield. Preliminary surveys conducted in Kerala showed the association of root-knot nematode and burrowing nematode in vetiver and lemon grass, the two important aromatic plants of the State (Sheela *et al.*, 1996).

### ORNAMENTAL PLANTS

The destructive plant parasitic nematodes are one of the major limiting factors in the production of ornamentals throughout the world. The narcissus industry of Britain, that was wiped out by the infestation of the nematode, *Ditylenchus dipsaci* stands testimony to this. Considerable growth reduction due to the nematode infestation has been reported on several plants like gladiolus, chrysanthemum and rose (Haque, 1972). Besides causing tremendous economic losses, the planting materials also act as carriers spreading the nematodes to newer areas.

In India, nematodes have emerged as economically important pests in major flower growing states like Karnataka, Tamil Nadu, West Bengal, Andhra Pradesh, Rajasthan and

Maharashtra. Incidence of *Meloidogyne* sp., *R. reniformis* and *Helicotylenchus* sp. were recorded from the rhizosphere of jasmine from different locations in Karnataka (Singh *et al.*, 1979). Bajaj, (1989) reported the incidence of the *Tylenchulus semipenetrans* in the rhizosphere of jasmine from Haryana. *M. incognita*, *M. javanica* and *M. arenaria* were identified as major limiting factors in the successful cultivation of tube rose in Tamilnadu and Karnataka (Babu and Vadivelu, 1988 and Khan and Reddy, 1922).

### Status of nematological research on ornamental plants in Kerala

The status of nematode as a pest of ornamental crops is practically unexplored in Kerala. Apart from the report of KAU (1997) on the incidence of root-knot and burrowing nematodes in anthurium and *M. incognita* in jasmine, hardly any report is available on nematode association in ornamental crops. With the inclusion of the State in the intensive floricultural zone for orchid, anthurium and other ornamental plants with immense export potential, it became imperative to identify the constraints in the cultivation of these crops. With this objective, studies were taken up to identify the important nematodes associated with ornamental plants and the extent of infestation in the state.

A random survey was conducted in Thiruvananthapuram district to gather information about the nematodes associated with cut flowers (anthurium and orchid), traditional flowers (rose and jasmine) and ornamental foliage plants (begonia and croton).

The root-knot nematode *M. incognita* and burrowing nematode, *R. similis* were found associated with these plants. Other nematodes observed were the lance nematode *Hoplolaimus* spp., spiral nematode

*Helicotylenchus* spp. and ring nematode *Criconeoides* sp. Among the cut flowers, anthurium was found to be highly susceptible to root-knot nematode. Small terminal galls were seen in anthurium, whereas comparatively large sized galls spread throughout the root system were visible in begonia and croton.

Histopathological studies were conducted in root knot infested anthurium, jasmine and begonia. In anthurium not much distortion could be distinguished in the stelar region in the root section of infested plants. Giant cells were seen close to the metaxylem of the xylem bundles, whereby the flow of food materials to the various parts of the plant was suppressed affecting the normal growth of the plant. No visible difference was detected between the phloem vessels in healthy and infested roots indicating that the phloem vessels were not affected by the nematode. Root section of the infested plants of jasmine showed signs of secondary growth. The innermost layer of cortex was well developed. Inner to that secondary phloem could be seen without damage or distortion. But the primary and the secondary xylem were completely destroyed and the central portion was completely occupied by few giant cells. The cortex was also seen slightly distorted. In begonia, the stelar region was completely distorted in nematode infested plants. The xylem bundles were unidentifiable, completely destroyed and were replaced by giant cells. Giant cell formation was observed from the third week after the inoculation of the nematode while dissolution of the cell wall was seen from fourth week onwards. The phloem vessels were not affected and the cortex was also much distorted.

Treatment of soil with carbofuran 2kg ai/ha gave maximum reduction of nematode population in anthurium. Neem cake 0.5 % w/w, carbofuran 1kg ai/ha + neem cake 0.25% w/w and carbofuran 1kg ai/ha + neem cake 0.25% w/w + *G. fasciculatum* 200 spores per pot were equally effective in reducing population of nematode in the soil. *G. fasciculatum* 200 spores/pot + neem cake 0.25 per cent w/w gave maximum reduction of nematode in root. A low gall index of 1.25 was observed in plants treated with carbofuran @ 2kg ai/ha and carbofuran @ 1kg ai/ha + neem cake 0.25 per cent w/w (Mahesh, 2001).

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# ENTOMOPATHOGENIC NEMATODES

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Extensive use of chemical pesticides has resulted in wide spread insect resistance to pesticides and adverse effect on beneficial insects, wild life and human life throughout the world. In response, there has been increased demand for alternative and selective pest control agents in particular, biological control. During the last decade there have been high lightened interest world over in the augmentative biological control of insects using entomopathogenic nematodes (EPN) belonging to the families, Steinernematidae and Heterorhabditidae. These nematodes are mutualistically associated with insect pathogenic bacteria in the genus, *Photorhabdus* / *Xenorhabdus*. Thus it is a nematode/ bacterium complex that work together as biological control unit to kill an insect host. These nematode bacterium association may meet many criteria for augmentative control of insect through inundative release including broad host range, ability to kill host rapidly, long duration storage of infective juveniles (IJ), distribution and persistence, available inexpensive mass production technologies and safety to plant, invertebrates and non target organisms and application with existing spray equipments, no known negative effect on the environment, amenable for genetic selection. These nematodes are

exempted from registration in many countries. The intense interest in EPN as biological control agent of insect pest has resulted in a large number of research efforts and subsequent publications.

## Biology of the nematode / bacterium complex

Steinernematids and heterorhabditids, obligate pathogens in nature, have the non-feeding, free living, third stage infective juvenile or dauer juveniles that infects the insect host in the soil environment. The infective juvenile, the only stage that occurs outside of an insect, is ensheathed in the second stage cuticle that is easily lost in the steinernematids but is retained in the heterorhabditids until just prior to or shortly after host infection. In addition, the nematode/bacterium association is highly specific. In the infective juvenile, the bacterial cells are housed in vesicle in the anterior part of the intestine for steinernematids and in the intestinal tract for heterorhabditids. The infective juveniles infect the host through natural openings (mouth, spiracles, anus) or thin areas of the host cuticle (common only in heterorhabditids) and penetrate into the host haemocoel. The mutualistic bacterium propagates and produces substance that

rapidly kill the host and protect the cadaver from colonization by other microorganisms. The nematode initiates its development, feeding on the bacterial cells and host tissues that have been metabolized by the bacterium and has one to three generations, depending on the host size. As the food resources in the host cadaver are depleted, a new generation of infective juveniles is produced and emerges from the host cadaver into the soil to search for new host.

A major difference between the steinernematids and heterorhabditids is that all but one species in the former group are amphimictic, whereas species in the later group are hermaphrodites in the first generation. Thus, steinernematids require a male and female infective juveniles to invade an insect host to produce progeny, whereas heterorhabditids need only one infective juvenile to penetrate and into a host as the resulting hermaphrodite adult is self-fertile.

The relationship between the nematode and bacterium is truly mutualistic for the following reasons. The nematode is dependent upon the bacterium for quickly killing its insect host, creating a suitable environment for its development by producing antibiotics that suppress competing microorganisms, transforming host tissue into food source and serving as food source.

The bacterium needs nematode for protection from the environment, penetration into host hemocoel and inhibition in the host antibacterial protein.

### **Mutualistic bacteria**

The bacteria mutualistically associated with EPN are *Xenorhabdus* and *Photorhabdus*

which are motile, gram negative, facultative, non spore forming, anaerobic rods in the family Enterobacteriaceae. In the genus *Xenorhabdus* five species are associated with *Steinernema* whereas in the genus *Photorhabdus* three species are associated with *Heterorhabditis* with one species *P. luminescens*, divided into five sub species. The subspecies of *P. luminescens* are subsp. *luminescens*, *laumondii*, *akhursti*, *kayaii* and *thraciaensis*. One species, *P. asymbiotica* has also been isolated from human clinical cases and is not associated with nematodes.

Major differences occur between the 2 bacterial genera. For example, most *Photorhabdus* spp. are luminescent and are catalase positive, whereas *Xenorhabdus* spp. have no luminescence and are catalase negative. Both bacteria produce phenotype variant cell types called primary form (phase I) and secondary form (phase II). The primary form is the cell type naturally associated with the nematodes whereas the secondary form can arise spontaneously when the bacterial cultures are in the stationary non-growth stage. The *Xenorhabdus* secondary form can revert to the primary form but this phenomenon has not been documented for *Photorhabdus* spp. Differences between primary form and secondary forms occur. The primary form produces antibiotics, absorbs certain dyes, and develop large scale intracellular inclusions, composed of crystalline proteins, whereas the secondary form does not or only weakly produces antibiotics, does not absorb dyes, and produces intracellular inclusions inefficiently. The primary form is superior to secondary form in its ability to support nematode propagation *in vitro*;



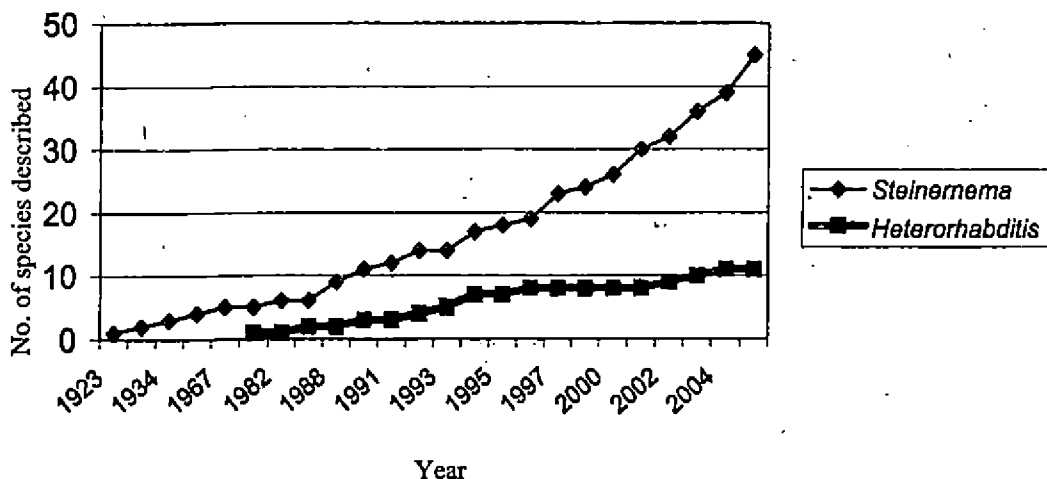
although some evidence suggests that this is not always the case. The reason for occurrence of the two forms is not known.

### Taxonomy of EPN

Over past several years, numerous changes have impacted EPN systematics. Earlier days, EPN were identified based on morphological characters which are overlapping and not stable in some species. The standardization of criteria for species description, proposal of name emendations, interpretation of phylogenetic relationships in the phylum Nematoda based on the molecular techniques, proposal of phylogenetic

species concept and new proposed classification of nematodes have brought the status of the EPN systematics into a phase of stability and growth which was now reflected in the number of species described in past 10 years (Fig. 1). At present, there are 57 recognized named species of EPN in the two families with 45 species in *Steinernema* one species in *Neosteinernema* and 11 species in *Heterorhabditis*. Though large number of EPN species have been described world wide only one species of *Heterorhabditis* (*H.indica*) and three species of *Steinernema* (*S.thermophilum*, *S.seemae*, *S.masoodi*) were described from India (Table 1).

Fig. 1 Total number of EPN described over the years



### Biogeography

Entomopathogenic nematodes have been recovered from soils from many parts of the world. Some nematodes appears to have a global distribution and are essentially ubiquitous. In India several surveys have

been conducted with the isolation of several known species (Table 2). The collection and identification of indigenous nematodes may provide isolates more suitable for inundative release against local pest species because of their adaptation to local climate.

Table.1. Distribution of entomopathogenic nematodes in India

Nematode	State	Reference
<i>H.bacteriophora</i>	Tamil Nadu	Sivakumar <i>et al.</i> , 1988
<i>H.indica</i>	Tamil Nadu	Poinar <i>et al.</i> , 1992 ; Josephraj Kumar and Sivakumar, 1997
<i>S.feltiae</i>	ICRISAT	Singh <i>et al.</i> , 1992
<i>Steinernema</i> sp. and <i>H.indica</i> <i>Steinernema</i> sp and <i>H.indica</i>	Tamil Nadu Tamil Nadu	Ambica , 1995 Josephraj Kumar and Sivakumar, 1997
<i>H.indica</i>	Kerala	Banu <i>et al.</i> , 1998
<i>S.abbasi</i> , <i>S.feltiae</i> , <i>S.bicornutum</i> , <i>S.carpocapsae</i> , <i>S.tami</i> , <i>S.glaseri</i> , <i>H.indica</i> and <i>H. bacteriophora</i> ,	Bangalore	Hussaini <i>et al.</i> , 1998, 2001, 2004 , 2005
<i>Steinernema</i> sp and <i>Heterorhabditis</i> sp	Gujarat	Vyas <i>et al.</i> , 1998
<i>S. thermophylum</i> and <i>Heterorhabditis</i> sp.	New Delhi	Ganguly and Singh, 2000
<i>Heterorhabditis</i> sp and <i>Steinernema</i> sp	Uttar pradesh & Himachal pradesh	Kaushal <i>et al.</i> , 2000
<i>H.indica</i> , <i>Steinernema</i> sp <i>S.siamkayai</i> and <i>S.glaseri</i> *	Kerala	Banu , 2001 Banu <i>et al.</i> , 2004; Banu <i>et al.</i> , 2005
<i>S.feltiae</i>	Punjab	Aujla <i>et al.</i> , 2001
<i>Heterorhabditis</i> sp	South and central zone	Gokte <i>et al.</i> , 2001
<i>Steinernema</i> sp and <i>Heterorhabditis</i> sp.	Rajasthan	Raj Kumar <i>et al.</i> , 2001; Parihar <i>et al.</i> , 2002
<i>Heterorhabditis</i> sp.	Andaman	Shyam Prasad <i>et al.</i> , 2001
<i>Steinernema</i> sp , <i>H.indica</i>	Kerala	Sosamma and Rasmi , 2001
<i>Steinernema</i> sp , <i>S.riobrave</i> and <i>Heterorhabditis</i> sp.	Gujarat	Vyas <i>et al.</i> , 2001
<i>H.indica</i> , <i>Steinernema</i> sp	Tamil Nadu	Sivakumar <i>et al.</i> , 2003 Uma Maheshwari , 2003
<i>H.india</i> <i>Steinernema</i> sp	Kerala	Banu <i>et al.</i> , 2004
<i>S.riobrave</i> , <i>S.asiaticum</i> , <i>H.indica</i>	Tamil Nadu	Chandrasekar <i>et al.</i> , 2004
<i>H.indica</i>	Karnataka	Praburaj <i>et al.</i> , 2005
<i>S. seemae</i> and <i>S.masoodi</i>	Kanpur	Ali <i>et al.</i> , 2005

\* *S. glaseri* was reisolated from the rhizosphere of cardamom where it was inoculated to control cardamom root grub, *Basilepta felvicone*

**Host range and safety to non-target organisms.**

Steinernematids and Heterorhabditids have been observed to infect over 200 species of insects from several orders. A few other arthropods are also susceptible to

infection. Report of susceptibility are often based on laboratory bioassays that are conducted under conditions favouring infection. The nematodes caused field epizootics in insect species only slightly susceptible under laboratory condition. The work done on EPN in India is summarized in Table 2.

Table 2. Host range of EPN in India

Nematode	Host tested	Laboratory/ Pot culture/ Field	Reference
1	2	3	4
<i>S.carpocarsae</i> (DD-136)	<i>Tryporyza incertulas</i>	Field	Rao <i>et al.</i> , 1971
<i>S.carpocapsae</i>	<i>Anomala</i> sp.	Field	Sundarababu <i>et al.</i> , 1984
<i>S.feltiae</i>	<i>Spodoptera litura</i>	Lab	Narayanan and Gopalakrishnan, 1987
<i>S.feltiae</i>	<i>S.litura</i> , <i>Helicoverpa armigera</i>		Lab Razak and Sivakumar, 1989
<i>S.carpocapsae</i> , <i>S.glaseri</i>	<i>Holotrichia consanguinea</i>	Lab.	Shanthi and Sivakumar 1991
<i>S.glaseri</i>	<i>H.consanguinea</i>	Lab	Vyas and Yadav, 1993
<i>S.carpocapsae</i> <i>H.bacteriophora</i> <i>Heterorhabditis</i> sp.	<i>Amsacta albistriga</i>	Lab	Baskaran <i>et al.</i> , 1994
<i>S.glaseri</i>	<i>H.armigera</i>	Lab	Patel and Vyas, 1995
<i>S.carpocapsae</i>	<i>S.litura</i>	Lab/Field	Sezhian <i>et al.</i> , 1996
<i>Steinernema</i> sp.	<i>Aproaerema modiella</i> <i>S.litura</i>	Lab/Field	Josephraj Kumar and Sivakumar, 1997
<i>H.indica</i>	<i>Rhynchophorus palmarum</i> , <i>Oryctes rhinoceros</i> , <i>Leucopholis coneophora</i> , <i>Opisina arenocella</i> , <i>Odoiporus longicollis</i>	Lab	Banu <i>et al.</i> , 1998
<i>S.glaseri</i> , <i>S.feltiae</i> , <i>H.indica</i>	<i>Chilo infuscatellus</i> , <i>C. sacchariphagus indicus</i> , <i>C. partellus indicus</i> <i>Sesamia inferens</i> , <i>S.litura</i> <i>H. armigera</i> , <i>Scirpophaga exerptalis</i>	Lab	Karunakar <i>et al.</i> , 1992 and 1999

1	2	3	4
<i>Steinernema</i> sp, <i>S.glaseri</i>	<i>S.litura</i> , <i>H.consanguinea</i>	Lab	Vyas <i>et al.</i> , 1999
<i>H. indica</i>	<i>P. americana</i>	Lab	Subramanian, 2000
<i>H. indica</i>	<i>Agrotis ipsilon</i>	Lab	Hussaini <i>et al.</i> , 2000
<i>S.glaseri</i> , <i>S.feltiae</i> , <i>H. indica</i>	<i>Holotrichia serrata</i> <i>Leucopholis lepidophora</i>	Lab	Karunakar <i>et al.</i> , 2000
<i>Heterorhabditis</i> sp, <i>Steinernema</i> sp	<i>Periplanata americana</i> <i>Acheta domesticus</i> <i>Odontotermes obesi</i>	Lab	Kaushal <i>et al.</i> , 2000
<i>S.glaseri</i> <i>Heterorhabditis</i> sp	<i>Myllocerus discolor</i>	Field	Praburaj <i>et al.</i> , 2000
<i>H.indica</i>	<i>P. americana</i>	Lab	Subramanian, 2000
<i>H.bacteriophora</i> <i>Heterorhabditis</i> sp <i>S.carpocapsae</i> <i>S.glaseri</i> <i>S.feltiae</i>	<i>P.xylostella</i>	Lab	Shinde and Singh, 2000, 2001, Singh and Shinde, 2002
<i>H.indica</i> <i>S.glaseri</i> <i>Steinernema</i> sp.	<i>Spodoptera litura</i> <i>Helicoverpa armigera</i> <i>Ergolis merione</i> <i>Eupterote mollifera</i> * <i>Sylepta derogata</i> <i>Leucinodes orbonalis</i> <i>Chilo sacchariphagus</i> <i>indicus</i> <i>Acherontia styx.</i> <i>O.rhinoceros</i> <i>R.ferrugineus</i> <i>Holotrichia serrata</i> <i>L. coneophora</i> <i>O. longicollis</i> <i>Cylas formicarius</i> <i>Mantis religiosa</i> <i>P.americana</i>	Lab	Banu, 2001; Banu and Rajendran, 2002
<i>Steinernema</i> sp.	<i>H. armigera</i>	Lab	Banu and Rajendran 2001
<i>S. carpocapsae</i> , <i>S.abbasi</i> , <i>S.tami</i> , <i>H.indica</i> , <i>S. bicornutum</i>	<i>A.ipsilon</i>	Lab	Hussaini <i>et al.</i> , 2001 and 2002
<i>Steinernema</i> sp. and <i>H.indica</i>	<i>R.palmarum</i> , <i>O.rhinoceros</i> , <i>L.coneophora</i> , <i>O. arenocella</i> , <i>O. longicollis</i>	Lab	Sosamma <i>et al.</i> , 2001

\* Insect mortality was recorded but nematode multiplication was not observed.

1	2	3	4
<i>H.indica</i>	<i>O. longicollis</i>	Lab	Padmanaban <i>et al.</i> , 2001
<i>H.indica</i>	<i>S. litura</i> , <i>P. xylostella</i>	Lab	Sharad Mohan <i>et al.</i> , 2001
<i>S.carpocapsae</i> , <i>S.abbsi</i> , <i>H.indica</i>	<i>A.ipsilon</i>	Lab	Hussaini <i>et al.</i> , 2002
<i>H.indica</i> , <i>H.bacteriophora</i> , <i>S.glaseri</i>	<i>Chilo infuscatellus</i>	Lab	Sankaranarayanan <i>et al.</i> , 2002
<i>H.minutus</i> , <i>S.glaseri</i>	<i>H.serrata</i> , <i>S.litura</i>		Praburaj <i>et al.</i> , 2002
<i>H.indica</i> , <i>H.bacteriophora</i> , <i>S.glaseri</i>	<i>S.litura</i>	Lab	Jothi and Mehta, 2002
<i>S.feltiae</i>	<i>H.armigera</i>	Field	Narayanan and Gopalakrishnan, 2003
<i>H.indica</i> , <i>S.glaseri</i> ,	<i>Helicoverpa armigera</i> , <i>Spodoptera litura</i> , <i>Eupterote mollifera</i> , <i>Dichocrocis punctiferalis</i> , [ <i>Conogethes punctiferalis</i> ], <i>Chilo sacchariphagus indicus</i> , <i>Cylas formicarius</i> , <i>Periplaneta americana</i>	Lab	Subramanian, 2000 and 2003
<i>S.riobrave</i> , <i>Heterorhabditis</i> sp.	<i>H.armigera</i>	Field	Vyas <i>et al.</i> , 2003
<i>H.indica</i> , <i>S.glaseri</i> , <i>Steinernema</i> sp	<i>R.ferrugineus</i>	Lab	Banu <i>et al.</i> , 2002
<i>Steinernema</i> sp , <i>H.indica</i>	<i>O.longicollis</i>	Lab	Banu <i>et al.</i> , 2002
<i>S.glaseri</i>	<i>Bombyx mori</i> , <i>H.serrata</i>	Lab	Praburaj <i>et al.</i> , 2003
<i>H.indica</i> , <i>H.bacteriophora</i> <i>S.carpocapsae</i> , <i>S.abbsi</i>	<i>Holotrichia longipennis</i>	Field	Hussaini <i>et al.</i> , 2004, 2005
<i>S.riobrave</i>	<i>H.consanguinea</i>	Field	Vyas <i>et al.</i> , 2004
<i>H.indica</i> , <i>S.glaseri</i>	<i>P. xylostella</i>	Glass house	Umamaheshwari <i>et al.</i> , 2004
<i>S.carpocapsae</i> , <i>S.riobrave</i> , <i>S.asiaticum</i> , <i>H.indica</i>	<i>Inderbella quadrinotata</i> , <i>Hypsipyla robusta</i> , <i>Hyblaea parea</i> , <i>Sahydrassus malabaricus</i>	Field	Chandrasekar <i>et al.</i> , 2004

1	2	3	4
<i>Steinernema sp.</i>	<i>A.ipsilon</i>	Green house	Mathasoliya <i>et al.</i> , 2004
<i>S.thermophilum</i> , <i>Steinernema sp.</i> , <i>H.bacteriophora</i>	<i>Boophilus microplus</i>	Lab	Rathour <i>et al.</i> , 2004
<i>S.thermophilum</i> , <i>S.glaseri</i> <i>Steinernema sp</i> , <i>H.bacteriophora</i> , <i>H.indica</i>	<i>Pierris brassicae</i>	Lab	Rathour and Ganguly 2004
<i>Steinernema sp.</i>	<i>Magaeselia sindhui</i>	Lab	Walia <i>et al.</i> , 2004
<i>S.feltiae</i>	<i>Bradysia tritici</i>	Lab	Aujla and Kaul., 2004
<i>S.riobrave</i>	<i>A.ipsilon</i>	Field	Mathasoliya <i>et al.</i> , 2004
<i>H.indica</i> , <i>H.bacteriophora</i> , <i>S.glaseri</i> , <i>S.riobrave</i>	<i>H. serrata</i>	Lab	Sankaranarayanan <i>et.al.</i> , 2004
<i>S.carpocapsae</i> (DD-136)	<i>Cnaphalocrosis medinalis</i>	Lab	Mohanty <i>et al.</i> , 2004
<i>S.abbasi</i> , <i>S.tami</i> , <i>S.carpocapsae</i> , <i>S.glaseri</i> , <i>H.indica</i>	<i>A.ipsilon</i>	Lab	Hussaini <i>et al.</i> , 2005
<i>H.indica</i>	<i>H.armigera</i>	Field	Praburaj <i>et al.</i> , 2005

### Research on Entomopathogenic nematodes at Central Plantation Crops Research Institute, Kasaragod.

In Kerala four surveys have been conducted with the isolation of three known species and several unidentified species. Banu *et al.*, 1998 first recovered *H.indica* from Kayankulam followed by the recovery of *H. indica* and *Steinernema sp.* by Sosamma *et al.*, 2001. Banu , 2001 did an extensive survey throughout Kerala and isolated 40 and 16 isolates of *H.indica* and *Steinernema sp.* respectively. A total of 597 samples were collected throughout Kerala. Entomopathogenic nematodes were recorded from 12 districts (85.71 %) out of 14 districts surveyed. They were recovered with a frequency of occur-

rence of 9.21 per cent (55/597). Heterorhabditids were found to occur more (6.70%) than Steinernematids (2.51%). In these districts surveyed highest frequency of occurrence of entomopathogenic nematodes was recorded in Ernakulam (16.00%) followed by Palakkad (10.00%). The frequency of occurrence of *Steinernema sp.* (6.67%) was high in Idukki district where as Ernakulam recorded the highest frequency of *Heterorhabditis sp.* (12.00%). The two nematode genera were not found occurring in same locality. Heterorhabditids were more ubiquitous than steinernematids, having been isolated from 12 districts out of 14 districts surveyed. Sample collected from the rhizosphere of cardamom yielded *S. glaseri*.

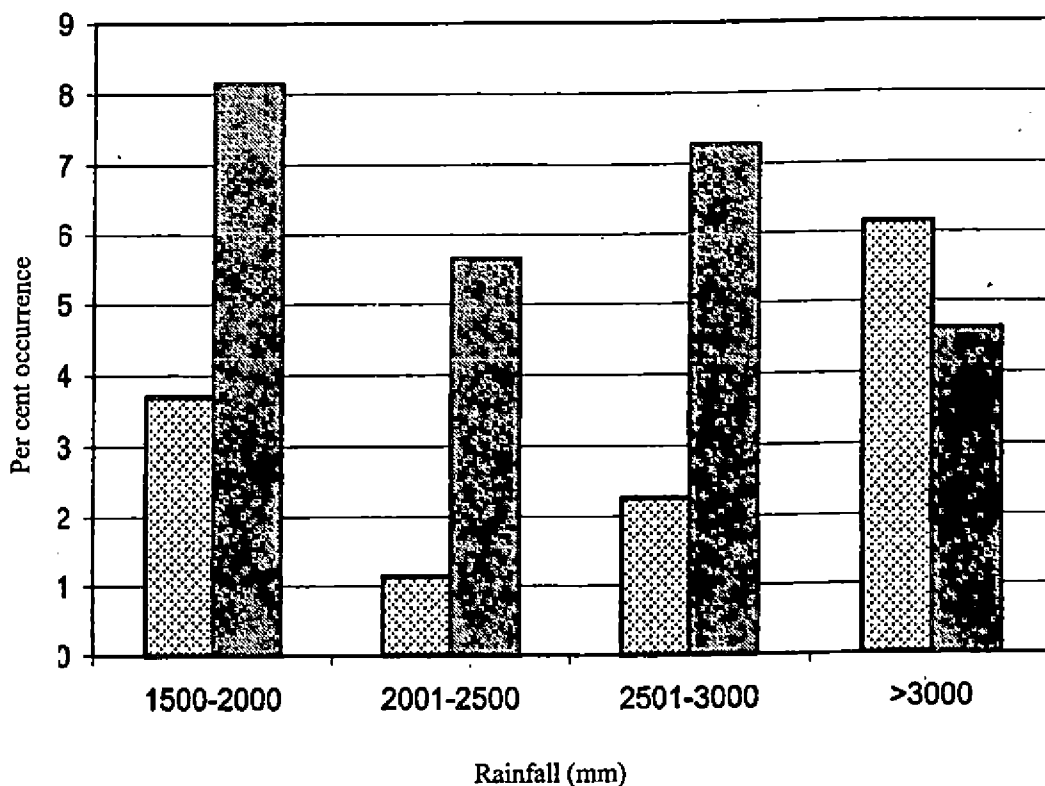
In first baiting, 50 samples (8.38%) were found to be positive for entomopathogenic nematode and the number of positive samples were increased to 55 (9.21%) in subsequent baiting. This clearly indicated that at least two baiting is essential to get maximum entomopathogenic nematode during survey. A survey conducted in white grub endemic areas of Kerala revealed the presence of a virulent strain of *Steinernema* sp. This was designated as isolate B1. Here EPN's were recovered less than 500m from seashore.

#### Rainfall

Entomopathogenic nematodes were

found to be present in areas with annual rainfall ranging from 1500 to more than 3000 mm. Steinernematids were recovered from 3.70, 1.13, 2.27 and 6.15 per cent samples collected from the localities receiving an annual rainfall of 1500-2000, 2001-2500, 2501-3000 and more than 3000 mm respectively. *Heterorhabditis* sp. occurred more in areas receiving annual rainfall of 1500-2000 mm (8.15%) followed by 2501-3000 mm (7.24%). Areas with annual rainfall ranging from 2001-2500 and more than 3000 mm recorded their occurrence with frequency of 5.65 and 4.62 per cent respectively (Fig. 2).

Fig. 2. Influence of rainfall on the occurrence of entomopathogenic nematodes



**Ecology  
Habitat**

Entomopathogenic nematodes were distributed in coastal, inland plain and hilly areas. Occurrence of *Heterorhabditis* sp. was more in inland plain (14.00%) followed by coastal area (7.72%). Hilly area recorded the highest occurrence (4.34%) of *Steinernema* sp. followed by coastal areas (2.90%) (Fig.3).

Both *Steinernema* sp. and *Heterorhabditis* sp. were found in different habitat like orchard with perennial fruit trees, plantations as well as agricultural fields grown with annual crops. Samples collected from annual crop showed the presence of *Steinernema* sp. and *Heterorhabditis* sp. in 1.76 and 6.34 per cent samples respectively and it was 2.50 and 4.70 per cent in perennial crops (Fig. 4).

Fig. 3. Occurrence of entomopathogenic nematodes in different areas

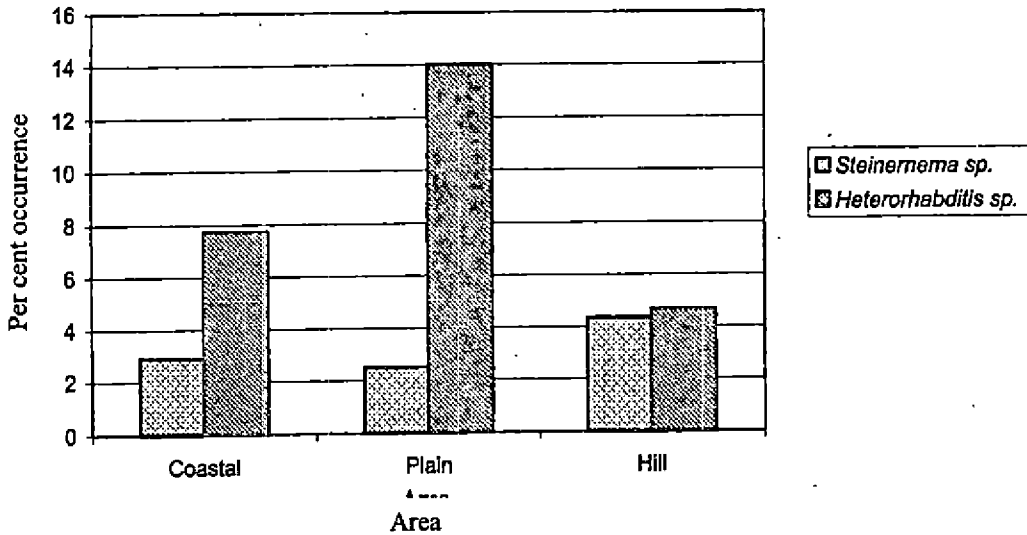
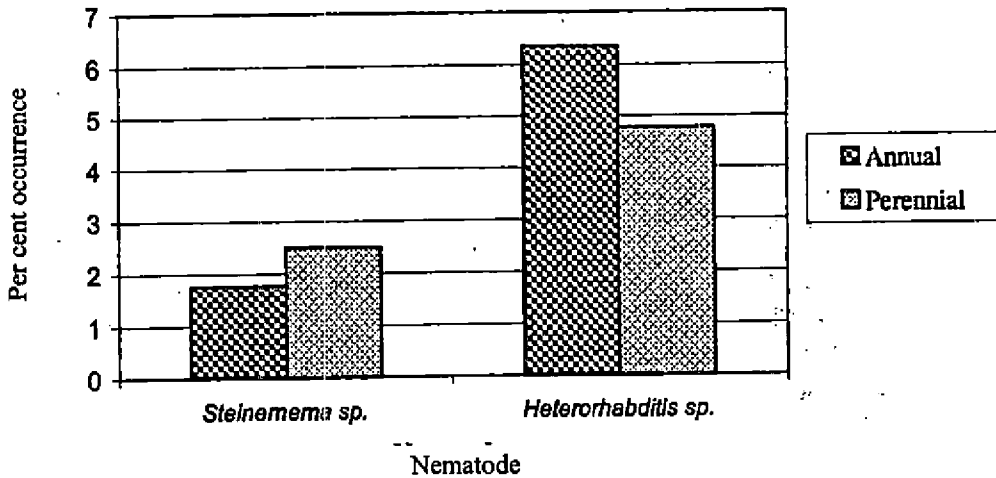


Fig. 4. Occurrence of entomopathogenic nematodes in different crops



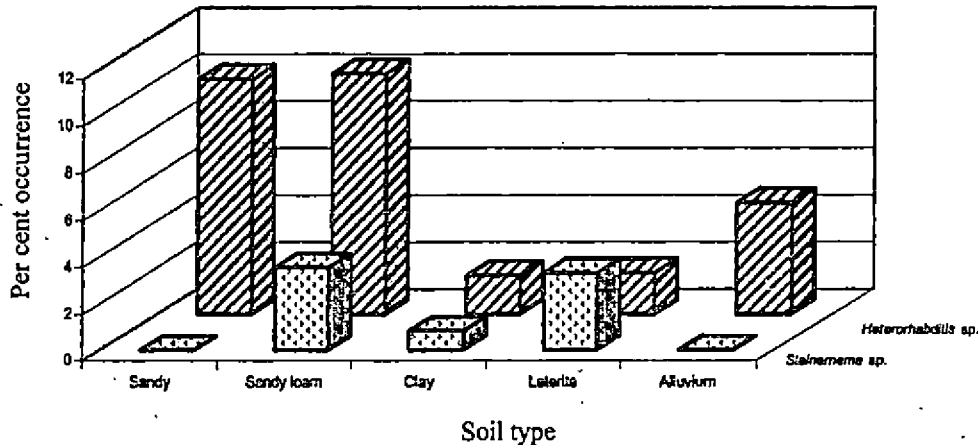


## Soil type

Soil type plays an important role in the occurrence and distribution of EPN. Soil type affects the survival and pathogenicity also. The occurrence of EPN was more in sandy loam soil followed by sandy soil and it was

low in clay soil (Fig.5) (Banu *et al.*, 2005). Generally higher clay content results in lower nematode survival which may be due to decreased pore size and reduced O<sub>2</sub> availability. Nematodes are generally more motile in sandy soil and the mobility decreased as the percentage of clay and silt increased.

Fig. 5. Occurrence of entomopathogenic nematodes in different types of soil



## Temperature

Temperature optima for infection and multiplication of three native EPN were studied at 15, 20, 25, 30 and 35°C. *H. indica* caused mortality of *G. mellonella* larvae in temperatures ranging from 15° to 30°C. Among the five temperatures tested, the optimum temperature for infection was 25°C as evidenced by lowest LC<sub>50</sub> value (4.28 IJ/larva). At 15°C, *H. indica* required 9.10 IJ/ larva to cause 50 per cent mortality. LC<sub>50</sub> at 20 and 30°C were 4.88 and 5.33 IJ/ larva respectively. No mortality of *G. mellonella* was recorded at 35°C. The optimum temperature for nematode multiplication was 25°C. Though insect mortality was observed at 15°C no nematode multiplication was recorded.

*S. glaseri* caused mortality of

*G. mellonella* at all the five temperatures. At 25°C *S. glaseri* was more virulent (LC<sub>50</sub> - 3.44 IJ/larva) which was followed by 30°C. At 15°C they were less virulent (10.56 IJ/ larva). *S. glaseri* multiplied in temperature ranging from 20 to 30°C. The maximum multiplication was recorded at 25°C which was followed by 20 and 30°C. Though insect mortality was recorded at 15 and 35°C, no nematode multiplication was observed.

Among the five temperatures tested for virulence of *Steinernema* sp. against *G. mellonella* was found to be more at 35°C (LC<sub>50</sub> - 3.60 IJ/larva). When the temperature decreased, the LC<sub>50</sub> value increased accordingly and it reached the highest of 8.87 IJ/ larva at 20°C. But at 15°C no mortality was observed. When nematode multiplication was compared, the highest of was recorded at 30°C.

## Storage

Between production and application, the infective juveniles are exposed to environmental stress especially during storage. Among the factors hampering the performance of entomopathogenic nematodes as biocontrol agents are the time and temperature conditions of storage. When three native entomopathogenic nematodes

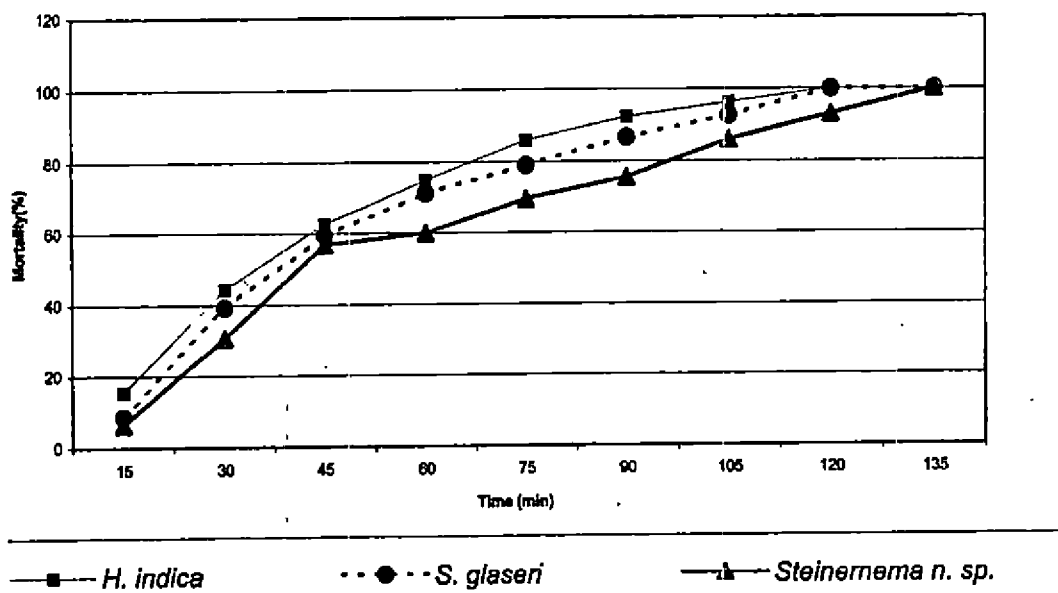
were stored at 15 and 25 ° C nematode survival and infectivity decreased over the period. Independent of time of storage, infective juveniles performed best when stored at 15 ° C. Survival and infectivity was decreased with increase in time of storage. For all three nematodes no difference in survival and infectivity was noticed up to 10 weeks of storage later it started decreasing (Banu, 2001).

## UV radiation

When EPN were applied to control foliar pests, they are directly exposed to UV radiation from sunlight. In general exposure to UV radiation rapidly reduced nematode pathogenicity and nematode exposed for longer duration were unable to cause lethal infection. Effect of UV radiation on the virulence of native EPN was studied under laboratory condition. The  $LT_{50}$  values for

*H.indica*, *S.glaseri* and *Steinernema* sp. on *G.melonella* larva was 33.51, 38.45 and 45.88 minutes / larva respectively. The result clearly indicated that the *Steinernema* sp. was more tolerant to UV radiation (Fig.6) (Banu and Rajendran, 2003). Addition of fluorescent brighteners is recommended to prevent nematodes from harmful UV radiation. The optical brighteners generally absorbs UV energy and convert it to visible light.

Fig. 6. Effect of UV radiation on *H. indica*, *S. glaseri* and *Steinernema* sp.



### **Mass multiplication under *in vivo* condition**

Influence of level of inoculum on the infectivity and multiplication of four native EPN (*H.indica* - two isolates, *S.glaseri*, *Steinernema* sp.) was studied in order to get maximum multiplication under *in vivo* condition. Density dependent factor within a host can have an important influence on the population dynamics of EPN. When different initial inoculum levels viz., 5,10,20,40,60 and 100 were tried on fully grown *G.melonella* larva, highest numbers of IJ per larva was harvested at an initial inoculum of 10 IJ/ larva and it was on par with 20 and 40IJ/larva for *H.indica* and *Steinernema* sp. (Banu, 2001) Nematode multiplication at lowest inoculum 5 IJ/larva was on par with 60and 100 IJ/ larva. Although the number of nematodes that established in the host increased with increase in dose, the percentage invasion decreased. For the success of EPN in the field the knowledge on the density effects of each species or strain should be taken into consideration.

Mass multiplication of native isolate of *Steinernema* sp (isolate B1) on third instar grub of *L. coneophora* was conducted under laboratory condition. The number of progeny produced initially increased with dose but the highest production (more than three lakh IJ / grub) was reached at an initial inoculum of 330 IJ/ grub. Time to first emergence of IJ was shorter when the number of IJ inoculated was larger. Effect of high density appears to be resulted from competition for limited nutrients within the host.

### **Mass multiplication under *in vitro* condition**

Mass multiplication of EPN was conducted under *in vitro* condition. Wouts

medium was supplemented with different locally available materials. Wouts medium supplemented with coconut oil for corn oil and green gram flour for soy flour recorded maximum nematode multiplication. The IJ's harvested were found to be more virulent also (Banu, 2001). Incorporation of coconut cake and coconut oil was found to increase the nematode multiplication under *in vitro* condition (Banu, Unpublished).

### **Compatibility of EPN with pesticides**

Chemical insecticides and EPN offer different but potentially compatible approach to suppress insect population. The EPN may seek out the host in inaccessible areas where pesticides may not act. A number of studies have shown that EPN's can survive exposure to various kinds of chemical pesticides.

Compatibility of commonly used pesticides viz., phorate, carbosulphan, nimbicidine and chlorpyrifos against management of white grub in coconut with native EPN (*Steinernema* sp B1) was tried under laboratory and field condition. Nematode mortality was increased with increase in period of exposure. *Steinernema* sp was compatible with all the pesticides under field recommended dose. In all the cases synergistic interaction between nematode and pesticides were recorded both in lab and field condition (Banu, Unpublished).

### **Pathogenicity**

Detailed pathogenicity studies on the native EPN against first, second and third instar of white grub under laboratory condition was carried out. Among three EPN (*Steinernema* sp. B1, *S. siamkayai* and *H.indica*), *Steinernema* sp. B1 recorded maximum mortality. A maximum of 3,00,000 IJ / grub was noticed at an initial inoculum of 330 IJ/ grub. All three EPN

multiplied well on white grub. When *Steinernema* sp. B1 was tried under green house and microplot condition significant decrease in grub population was noticed (Banu, Unpublished).

**Research on Entomopathogenic Nematodes at Central Plantation Crops Research Institute, Kayamkulam**

Soil surveys conducted in Kerala

indicates wide spread occurrence of *Heterorhabditis indica* in sandy loam soils (128 out of 430 samples) while *Steinernema* sp. was obtained from only three samples. (Tables 1, 2). They could be stored in foam pieces in distilled water at 10°C up to eight months without affecting their viability.

Table 1. Soil survey of EPN in Kerala

District	No. of samples collected	No. of samples positive for EPN		
		<i>H. indica</i>	<i>Steinernema</i>	Total
Alappuzha	217	56	2	58
Kottayam	20	4	-	4
Palakkad	67	15	-	15
Thiruvananthapuram	21	7	-	7
Thrissur	31	14	-	14
Kozhikode	43	18	-	18
Wayanad	25	3	1	4
Pathanamthitta	26	11	-	11
Total	430	128	3	131

Table 2. Distribution of EPN in different soil types

Soil type	No. of samples collected	No. of samples positive	
		<i>Heterorhabditis indica</i>	<i>Steinernema</i> sp.
Laterite	138	44	-
Alluvial	39	10	-
Sandy loam	255	69	3
Clay	36	5	-

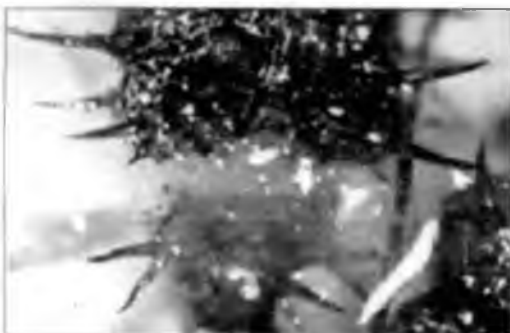
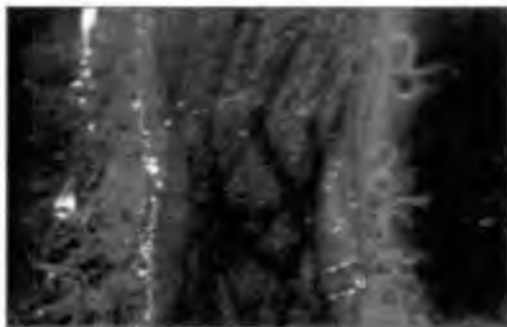
Effect of neem leaf extract on EPN isolates was studied by treating the infective juveniles (IJ<sub>s</sub>) with extract for 24 h and subsequently studying their infectivity to *Corcyra caphalonica* larvae. The IJ<sub>s</sub> remained alive and produced larval mortality in two days.

The effect of temperature on infectivity and growth of *H. indica* on *Corcyra* larvae at 4 different temperatures, 10 to 12, 16 to 18, 20-24 and 28-30°C was studied. The fastest insect mortality was recorded at 22-24°C followed by 28-30°C (Sosamma, 2003).

## EPN infestation in crop pests



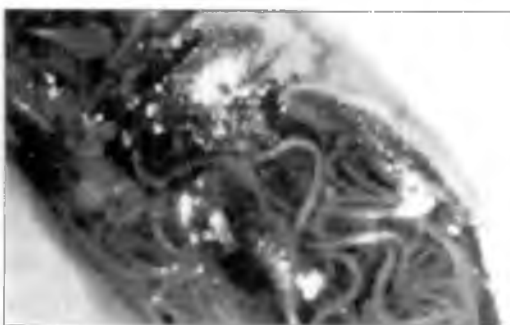
Rice bug (*Leptocorisa acuta*)



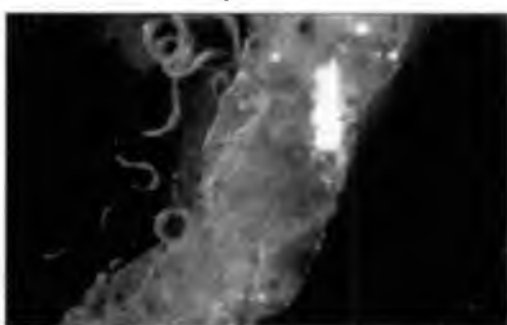
Rice hispa (*Dicladispa armigera*)



Leaf roller (*Cnaphalocrocis medinalis*)



Blue beetle (*Haltica cyanea*)



Fruitfly maggot (*Bactrocera cucurbitae*)



*Leptoglossus australis*





## 1. Rhinoceros beetle (*Oryctes rhinoceros*.L)

Kurian *et al.*, (1967) first isolated nematodes from third instar grubs of Rhinoceros beetle which when injected into healthy grubs produced mortality. DD-136 was found to be effective against larval stages. DD-136 was found to remain viable in absence of any biological hosts in cattle dung even after 12 months. Hoy (1954) reported the use of entomopathogenic nematode *Steinernema glaseri* against *O. rhinoceros* in Wallis Island. *Heterorhabditis* species have been reported to occur in diseased *O. rhinoceros* grubs and found pathogenic to it. First instar larvae of *O. rhinoceros* were found to be resistant to steinernematid infection (Zelazny, 1985). *Rhabditis* sp. has also been reported from *O. rhinoceros*. Prasad and Raghunath (2000) and Sosamma and Rasmi (2001) reported the occurrence of *Thelastoma* sp. on *O. rhinoceros*. Since EPN is effective against soil dwelling stages of pests, spraying infective juvenile suspension in breeding site is recommended for effective control of pests. Since the temperature at these sites is very high (40°C), nematodes cannot survive unless some moisture is always retained which is not practical under field conditions. Hence temperature - tolerant strains against such pests are to be evolved and tested.

*Heterorhabditis* are probably superior against scarabs and other soil insects because they are active, move down in the soil profile and apparently can penetrate the soft inter segmental membrane of insects (Gaugler and Kaya, 1990). Pathogenicity trials at Central Plantation Crops Research Institute indicate that *H. indica* isolate is more virulent to *O. rhinoceros* than *Steinernema*. When a mixture of *H. indica*

and *Steinernema* sp. was treated against *O. rhinoceros* grubs in earthen pots, only *H. indica* could be recovered from the dead cadavers. *Steinernema* sp. was found to be less efficient against *O. rhinoceros* in the laboratory trials also. (Sosamma, 2003).

## 2. Red palm weevil (*Rhynchophorus ferrugineus*)

Red palm weevil *Rhynchophorus ferrugineus* is a fatal enemy of palms, particularly during the early periods of its growth. The grubs feed on the tender portions inside the palm crown. A wide range of palms is recorded as host plants of red palm weevil.

Cryptic behaviour of all developmental stages of the insect can make the control of red palm weevil very difficult. However the EPN remains best possible means of controlling this pest. *Steinernema abbasi* and *H. indica* were isolated from red palm weevil from date palms (Abbas *et al.*, 2001), and were found to be virulent. Various modes of application of EPN against red palm weevil have been tried. Results were not encouraging when infective juveniles are injected in to the stem of palms. This may be due to the deleterious effect of frass on the nematode. Abbas *et al.* (2000) reported that the adults of red palm weevil inhabit soil occasionally seeking shade and shelter so that control, can be affected by soil application of EPN. Isolation of EPN from adult stages of the weevil is also attributed to this fact. Crown application of EPN using antidesiccants was found to enhance insect mortality (Abbas *et al.*, 2000). The application of EPN to leaf axils of palm trees is also recommended. (Sosamma, 2003)

### 3. Root grub

(*Leucopholis coneophora* Burm.)

Against root grubs, applying EPN through trickle or drip irrigation can be tried.

### 4. Leaf eating caterpillar

(*Opisina arenosella* Walker)

Against foliar pest *Opisina arenosella* EPN was found to be effective in lab. Field application is yet to be tried.

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### Paddy

A survey was conducted for entomopathogenic nematode (EPN) on paddy crop and 10 isolates belonging to the genus *Heterorhabditis* were isolated and found to be pathogenic on the pests of rice like rice bug, *Leptocorisa acuta* (Thumb); brown plant hopper, (*Nilaparvatha lugens* (Stal.); rice hispa, (*Diuraphis* spp.); grass hopper (*Heteroglyphus banian* (Fab.); small rice grass hopper, *Oxya chinensis* (Thunberg); green leaf hopper, *Nepotettix* spp., rice leptispa, *Leptispa pygmaea* (Baly.); gregarious blue beetle, *Haltica cynea* (Web.). They are also pathogenic to gram pod borer, *Heliothis armigera*; semilooper, *Achoea janata*; tobacco caterpillar, *Spodoptera litura*.

### Vegetables

Effect of two native isolates of *Rhabditis* was assessed in terms of dipteran and coleopteran pests of bitter melon namely fruit fly, *Bacterocera cucurbitae* Coq. and epilachna beetle, *Epilachna septima* Fab. The native isolates from the rhizosphere of vegetables using wax moth, *Galleria melonella* larvae as trap were used for assessing the mortality of maggots of

*B. cucurbitae* and grubs of *E. septima* of bitter melon. Active third stage juvenile (dauer juveniles) stored for seven days in sterile tap water were used for inoculation following standard methods. Three levels of native isolates of *Rhabditis* were inoculated (50, 100 and 200 DI/maggot or grub). The results revealed that the mortality of *B. cucurbitae* ranged from 6-47 % at an inoculum level of 50, 100 and 200 DI/insect larvae. At the highest dose 87 % mortality was recorded at 48 h after treatment and it became cent percent at 72 h after treatment. The mortality of grubs of epilachna beetle due to infestation by isolates of *Rhabditis* ranged from 67-87% at 48 h after treatment. At 72 h after inoculation, all the isolates showed cent percent mortality. The nematode multiplication was seen in insect cadaver 72 h after treatment.

Effect of two isolates of entomopathogenic nematode, *Heterorhabditis* sp. was assessed in terms of larval mortality of leaf eating caterpillars of vegetables viz., *Sylepta derogata* L. (Pyralidae: Lepidoptera), *Diaphania indica* Saund. (Pyralidae: Lepidoptera) and *Spilosoma oblique* W. (Arctiidae: Lepidoptera). All the three pests showed high mortality after 48 h of inoculation (40-100%). Based on the results of the study, mortality of the above three larvae were comparatively higher in *Heterorhabditis* isolate (2) at 24 and 48 hours after inoculation while the effect of *Heterorhabditis* isolate (1) was found superior in all the above pests at 72 inoculation. However, the mortality at 24, 48 and 72 h varied depending upon the isolate and insect pest studied revealing the potential of these nematodes as biocontrol agents against lepidopteran pests (Sheela *et al.*, 2002).



## Arecanut

One entomopathogenic nematode collected locally and identified up to generic level as *Heterorhabditis* sp. has been screened against the arecanut spindle bug (*Carvalhoia arecae* Miller) under laboratory conditions in petri plates. The nematode entered inside the bug and caused its mortality within 24 – 72 h of treatment. Adult females and males along with juveniles were found in large numbers in insect body indicating that they have grown into adult and multiply inside the insect host. Nematodes were found to have symbiotic association with a bacterium that was found inside the oesophagus and intestinal lumen in large numbers. Infective third stage juveniles of the nematode cultured on live *Corcyra cephalonica* brought about 90% mortality of larvae and adult bugs 48 h after treatment and cent percent after a week. The treatments remained effective for 3 - 4 months after which the palms were found to have fresh attacks by insects from neighbouring fields.

## Sweet potato

Mohandas *et al.* 2004 reported a pathogenic species of *Rhabditis* (oscheius) from grubs and pupae of sweet potato weevil with ability to kill many insect pests (Hemipterans and Lepidopterans).

## Future thrusts

Isolation of more and more native EPN from different states of the country with search for virulent strains/ species for different groups of insects which are having adaptation to local climates and also ecologically well compatible. Development of easy and cheap mass production/ fermentation

technique with suitable formulations for commercialization of indigenous isolates. Low cost industrial mass production technique with greater virulence of augmented EPN. Genetic improvement of native isolates for greater virulence and desiccation tolerance. Biotechnological studies are to be strengthened on symbiotic bacteria and their genetics for development of transgenic plants in near future.

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