Nema III(813)

INVESTIGATIONS ON NEMATODES ASSOCIATED WITH SPICES

Final Report

Compiled and Edited by
Santhosh J. Eapen

2006

iisr
INDIAN INSTITUTE OF SPICES RESEARCH
(Indian Council of Agricultural Research)
Calicut - 673012, Kerala

CONTENTS

PART I: GENERAL INFORMATION

800 Project Code

8001 Institute Project Code No. : Nema III (813)

8002 ICAR Project Code No. : -

801 Name of the Institute and Division

8011 Name and address of Institute: Indian Institute of Spices Research, Calicut – 673012, Kerala

8012 Name of Division / Section : Crop Protection/Nematology

8013 Location of the Project : IISR, Calicut

802 Project Title : **INVESTIGATIONS ON NEMATODES ASSOCIATED WITH SPICES**

(Formerly 'Investigations on Nematodes Associated with Ginger, turmeric and Black Pepper')

803 Priority Area :

8031 Research Approach :

Applied Research	Basic Research	Process/Technology development	Transfer of Technology
01	02	03	04

804 Specific Area : **PLANT NEMATOLOGY**

805 Duration of Project :

8051 Date of start : 1991

8052 Date of Completion : 2006

806 Total cost /Expenditure Incurred : Rs. 33 lakhs

(Give reasons for variation, if any from original estimated cost)

- Extension of the project
- Due to escalation in costs
- Change in the scope of the project

807 EXECUTIVE SUMMARY

Nematode problems of ginger and turmeric were studied in this project. Surveys conducted in ginger fields of Ernakulam, Kottayam, Idukki, Kozhikode and Wynad districts of Kerala by collecting around 250 samples revealed association of seven genera of plant parasitic nematodes. In another survey conducted in Adilabad, Nizamabad and Karimnagar districts of Andhra Pradesh, six genera of plant parasitic nematodes were recorded on turmeric. In both the surveys, the predominant species were *Meloidogyne* sp. and *R. reniformis*. *M. incognita* caused significant reduction in growth and yield of four turmeric varieties viz. Suvarna, Suguna, Sudarshana and Alleppey. In ginger, M. incognita caused considerable reduction in growth parameters at Pi<2/100 cc soil. Interaction studies with *M. incognita* and *Pythium aphanidermatum* clearly established the role of *M. incognita* in predisposing ginger plants to soft rot disease. Plants pre-inoculated with the nematode succumbed to the disease earlier to plants infected with the fungus alone. However, nematodes had no role in bacterial wilt which was primarily due to infection by *Ralstonia solanacearum*.

Attempts were also made to study the variability in root-knot nematodes by studying their variation in perineal and isozyme patterns. For this, 36 populations of root-knot nematodes were collected from different spice crops grown in different parts of Kerala, Karnataka, Andhra Pradesh and Tamil Nadu. Considerable variability in perineal patterns was noticed in several populations. Variability in esterase and malate dehydrogenase isoenzyme patterns was also observed in some of the populations studied. A new root knot nematode species, *M. piperis*, infesting black pepper was identified from Peruvannamuzhi.

Genomic DNA was isolated from *M incognita* and *Radopholus similis* for the first time. Amplification of rDNA viz. 18S and D2-D3 expansion region, was achieved by using two universal primers. Species-specific primers were designed for *Radopholus* and *Pratylenchus* based on sequence information from the ITS gene using Bioinformatics tools. The primer sets generated a single PCR fragment of 398 bp that was specific to *R. similis*. The amplified product was cloned, sequenced and the sequence was deposited in the nucleotide sequence database of EMBL (AM286692).

Inoculation of black pepper cuttings with a nematophagous fungus *Paecilomyces lilacinus* suppressed infestation by *M.incognita* and *R.similis*. Incorporation of green leaves of *Strychnos nuxvomica* and *Piper colubrinum* in basins of black pepper vines reduced the foliar yellowing due to nematode infection. Vermicompost amendment had no effect on nematodes. In vitro evaluation of commercially available neem products indicated that 'Econeem' was highly toxic to root-knot nematodes at 2 ml/l or above.

808 Key words : Genetic diversity, *Radopholus similis*, *Meloidogyne incognita*, *Meloidogyne piperis*, root knot nematodes, rDNA, isozymes, *Strychnos nuxvomica*, *Piper colubrinum*

PART II : INVESTIGATORS' PROFILE

810 Principal Investigator :

81011	Name	:	**K.V. Ramana** (1991-03)
81021	Designation	:	Principal Scientist & Head
81012	Name	:	**Santhosh J. Eapen** (2003-06)
81022	Designation	:	Senior Scientist (Nematology)
8103	Division/ Section	:	Division of Crop Protection
8104	Location	:	IISR, Calicut
8105	Institute Address	:	P.B. No. 1701, Marikunnu Post Calicut – 673 012, Kerala

811 Co- Investigators:

81111	Name	:	Santhosh J. Eapen (1994-03)
81121	Designation	:	Scientist Sr. Scale (Nematology)
8113	Division/ Section	:	Division of Crop Protection
8114	Location	:	IISR, Calicut
8115	Institute Address	:	P.B. No. 1701, Marikunnu Post Calicut – 673 012, Kerala

PART III: TECHNICAL DETAILS

820 Introduction and objectives

8201 Project objectives

- To identify plant parasitic nematodes associated with spice crops and to understand the nature of damage to the crops.
- To identify economically important nematode species
- To develop nematode management strategies to boost up the yields of spice crops.

8202 Background information and importance of the project

Nematodes are roundworms, and those that attack plants are microscopic. The damage they cause to plants is often subtle and is easily confused with nutrient problems. Although hundreds of different kinds of nematodes may infect plants, less than a dozen are economically serious root-feeding pathogens on spices, and only three genera viz. *Meloidogyne*, *Radopholus* and *Pratylenchus* causes significant damage to spices. Out of these, nematode problems of black pepper and cardamom were studied in separate projects. Basic studies on major nematodes and exclusive studies on nematode problems of ginger and turmeric were carried out under this project.

Ginger and turmeric are important spice crops grown in India. Ginger is widely grown in Kerala which accounts 43% of total production and turmeric is grown on a large scale in Andhra Pradesh. As many as 10 genera of plant parasitic nematodes have been reported with the crops out of which root-knot nematodes are major problems resulting in crop loss up to 46%. Besides root-knot namtodes, *R. similis* and *Pratylenchus* sp are alsoreported on these crops. In view of their association with these crops, it is necessary to ciduct a systematic survey to identify plant parasitic nematodes associated with these crops in the major growing areas, assess the crop loss, identify resistant/ tolerant culticars and to develop suitable nematode management strategies in ginger and turmeric.

821 Project Technical Profile

8211 Technical programme
(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environments etc.)

- Survey for plant parasitic nematodes associated with ginger and turmeric.
- Establish pathogenecity of important nematode species on ginger and turmeric and assess their damage potential.
- Screening germplasm of ginger, turmeric and black pepper to important plant parasitic nematodes.

- Study the role of plant parasitic nematodes, if any in the disease complex in ginger.
- Test the efficacy of nematicide and bio control agents in suppressing nematode infestation.
- Population variability in root-knot nematodes associated with spices
- Isozyme analysis of different root-knot nematode populations of spice crops
- Role of *M. incognita* in the rhizome rot complex of ginger
- Effect of different mulches on major nematodes of black pepper and ginger

8212 Total man months involvement of component project workers

80 man months

822 Final Report on the Project

Detailed report containing all relevant data with a summary of results

8221 Achievements in terms of targets fixed for each activity

Please see Annexure I.

8222 Questions answered

8223 Process/ Product/ Technology/ Developed

8224 Practical Utility
(not more than 150 words)

Though India is traditionally considered as the 'land of spices', productivity of many spices is very low. This is mainly because of the damage caused by pests and pathogens including nematodes. Despite the fact that chemical control methods are effective, they are less preferred because they are cost prohibitive. Environmental and human health concerns have led to increased restrictions on the use of nematicides. Thus in future nematicides are likely to have a diminishing role in crop protection. Besides Integrated Pest Management (IPM) strategies compatible with organic agriculture are the need of the hour.

- Considering the role of root-knot nematodes in soft rot disease of ginger, a nematode management component has to be included in the disease management package.
- The root-knot nematode resistant ginger and turmeric lines can be tested further in hot spot areas and if found resistant can be recommended for cultivation in areas where these nematodes are prevalent.
- The experiment to evaluate the usefulness of organic mulches, if found

successful, will help to formulate a non-chemical nematode management option.

- Considering the role of root-knot nematodes in soft rot disease of ginger, a nematode management component has to be included in the disease management package.
- Some of the promising root-knot nematode resistant ginger and turmeric lines can be tested further in hot spot areas and if found resistant can be recommended for cultivation in areas where these nematodes are prevalent.
- The experiment to evaluate the usefulness of organic mulches, if found successful, will help to formulate a non-chemical nematode management option.

8225 Constraints, if any

- Poor awareness among the growers regarding the need for nematode control
- Lack of information on the genetic diversity of major nematodes infesting various crops in India.

823 PUBLICATIONS AND MATERIAL DEVELOPMENT

8231 Reviews

a. Ramana, K.V. 1991. Slow decline disease of black pepper (*Piper nigrum* L.) in India. In *Diseases of Black Pepper* (Eds.). Y.R. Sarma and T. Premkumar. National Research Centre for Spices, Calicut, pp. 136-157.

b. Sarma, Y.R., Anandaraj, M. and Ramana, K.V. 1992. Status of slow decline disease of black pepper and approaches of disease management. In: *Proc. of The International Workshop on Black Pepper Diseases* (Eds. Pasril Wahid, D. Sitepu, S. Deciyanto and Ujang Suparman) Bandar Lampung, Indonesia, pp. 167-173.

c. Sarma, Y.R., Anandaraj, M. and Ramana, K.V. 1992. Present status of black pepper diseases in India and their management. In: *Proc. of The International Workshop on Black Pepper Diseases* (Eds. Pasril Wahid, D. Sitepu, S. Deciyanto and Ujang Suparman) Bandar Lampung, Indonesia, pp. 67-76.

d. Ramana, K.V. and Santhosh J. Eapen, 1992. Plant parasitic nematodes of black pepper and cardamom and their management. In *Black Pepper and Cardamom: Problems and Prospects* (Eds Y.R. Sarma, S. Devasahayam and M. Anandaraj), Indian Society for Spices, Calicut. pp. 43-47.

e. Ramana, K.V., Sarma, Y.R. and Anandaraj, 1992. Nematode management in black pepper. In *The Pepper Industry, Problems and Prospects* (Eds. M.Y. Ibrahim, C.F.J. Bong and I.B. Ipor) Universities Pertanian Malaysia, Bintulu Campus, Bintulu, Sarawak, Malaysia. pp. 118-131.

f. Ramana, K.V. and Santhosh J. Eapen, 1994. Parasitic Nematodes and their management in major spices. National Seminar on Diseases of Spices, Indian Society for Spices, Calicut, 7-8 April, 1994.

g. Ramana, K.V. and Santhosh J. Eapen 1995. Parasitic nematodes and their management in major spices. *J. Spices & Aromatic Crops* 4: 1-16.

h. Ramana, K.V. and Santhosh J. Eapen 1995. Nematode problems in spices and condiments. In *Nematode Pest Management - an Appraisal of Eco-friendly Approaches.* Pp. 263 - 270. Ed. G. Swarup, D.R. Dasgupta & J.S. Gill. Nematological Society of India, New Delhi, India.

i. Ramana, K.V. and Santhosh J. Eapen 1998. Plant parasitic nematodes associated with spices and condiments. In *Nematode Diseases in Plants.* Pp. 217 – 251. Ed. P.C. Trivedi. CBS Publishers and Distributors, New Delhi.

j. Ramana K V and Santhosh J Eapen 1999. Integrated nematode management in spice crops. In *IPM System in Agriculture - Volume 6 Cash Crops*, Eds. R.K. Upadhyay, K.G. Mukherji and O.P.Dubey, pp. 381 – 399. Aditya Books Pvt. Ltd., New Delhi, India.

k. Ramana, K.V. and Santhosh J. Eapen 1999. Nematode induced diseases in black pepper. In *Black Pepper – A Monograph.* Pp. 269 – 295. Ed. P.N. Ravindran. Harwood Academy Publishers, Amsterdam, The Netherlands.

l. Ramana, K.V. and Santhosh J. Eapen 1999. Nematode pests of spices and their management. *Indian Journal of Arecanut, Spices and Medicinal Plants* 1(4): 146-153.

m. Kaloo, G., Pal. R. N., Ramana, K. V. and Sarma, Y. R. 2001. Vistas in spices research – An overview of 25 years of spices research achievements at IISR. In: Spices Indica – Silver Jubilee Souvenir Indian Institute of Spices Research pp. 6-23 (Eds.) Y. R. Sarma, B. Sasikumar and B. Chempakam. Indian Institute of Spices Research, Calicut, India.

n. Ramana, K.V. and Eapen, S.J. 2001. Nematode diseases of spices and condiments and their management. *National Congress on Centenary of Nematology in India*. December 5-7, 2001, Indian Agricultural Research Institute, New Delhi.

o. Ramana, K.V. and Eapen, S.J. 2002. Nematode diseases of black pepper. '*Nema 100 Kerala*'. February 21-22, 2002. Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala.

p. Eapen, S.J. and Ramana, K.V. 2002. Nematode diseases of ginger, turmeric and tree spices. '*Nema 100 Kerala*'. February 21-22, 2002. Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala.

q. Ramana, K.V. and Eapen, S.J. 2002. Nematology Research at Indian Institute of Spices Research. In: *Milestones of Nematological Research in Kerala*, Pp. 36 - 50. (Eds.) P.K. Koshy, V.K. Sosamma & Gulsar Banu. Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala.

r. Koshy, P.K., Santhosh J. Eapen and Rakesh Pandey 2005. Nematode Parasites of Spices, Condiments and Medicinal Plants. In: *Plant Parasitic Nematodes in Tropical and Subtropical Agriculture* -2 Edition. Pp. 751-791. Eds. M. Luc, R.S. Sikora and J. Bridge, CAB International, Wallingford, U.K. (CABI Publishing).

s. Santhosh J. Eapen 2005. Nematodes of Spices. In: *Nematode Pests of Crops in Kerala- An Overview,* Pp 63-86. (Ed.) Sheela M.S. et al., Kerala Agricultural University.

t. Santhosh J. Eapen 2006. 'Diversity of parasitic nematodes and their antagonists in spices'. In: *National Conference on Agro-biodiversity*, 12-15 Feb. 2006, National Biodiversity Authority of India, Chennai.

8232 Research papers

a. Mohandas, C. and Ramana, K.V. 1991. Pathogenecity of *Meloidogyne incognita* and *Radopholus similis* on black pepper (*Piper nigrum* L.) *J. Plant Crops,* 19: 41-53.

b. Sarma, Y.R. Anandaraj, M. and Ramana, K.V. 1991. Role of *Phytophthora* and parasitic nematodes in root rot of black pepper (*Piper nigrum* L.) in slow decline disease. *Phytophthora Newsl.* 17: 42-43.

c. Ramana, K.V., Sarma, Y.R. and Mohandas, C. 1992. Slow decline of black pepper (*Piper nigrum* L.) and role of plant parasitic nematodes and *Phytophthora capsici* in the disease complex. *J. Plant. Crops* 20 (suppl.): 65-68.

d. Ramana, K.V. 1994. Efficacy of *Paecilomyces lilacinus* (Thom) Samson in suppressing nematode infestation in black pepper (*Piper nigrum* L.). In *J. Spices and Aromatic Crops* 3: 130-134.

e. Anandaraj, M., Ramana, K.V. and Sarma, Y.R. 1994. Role of *Phytophthora capsici* in the etiology of slow decline disease of black

pepper. International Symposium on Plantation Crops (PLACROSYM XI), Calicut, 30th November – 3rd December, 1994. (Abstract) p.23.

f. Eapen S. J. 1995. A note on the incidence of *Rotylenchulus reniformis* in cardamom plants. Indian J. Nematol. 25: 213 – 214.

g. Ramana K V, Eapen S J and Sarma Y R 1998. Effect of *Meloidogyne incognita, Pythium aphanidermatum* and *Ralstonia solanacearum* alone and in combinations in ginger. In: Nematology: Challenges and Opportunities in 21st Century, (Ed.) Usha K Mehta. Afro-Asian Society of Nematologists, Luton, England. Pp.87-92.

h. Sahoo, N.K., Sudershan Ganguly and Santhosh J. Eapen 2000. Description of *Meloidogyne piperi* sp.n. (Nematoda: Meloidogynidae) isolated from the roots of *Piper nigrum* in South India. Indian Journal of Nematology 30: 203 – 209.

8233 Popular articles

a. Sarma, Y.R., Anandaraj, M., Venugopal, M.N., Suseela Bhai, R., Rajan, P.P., Ramana, K.V. and Santhosh J. Eapen 1996. Eco-friendly disease management strategies in spice crops. *The Planters' Chronicle* 91: 15-18.

b. Santhosh J. Eapen 1999. Pests and diseases of ginger and turmeric (Mal.). Karshakasree 4(9): 41

c. Santhosh J. Eapen 2003. Nematodes - the hidden enemies (Mal.). *Kerala Karshakan* 49(1): 23-24, 26.

8234 Reports

a. Srinivasa Rao Boyina 2004. *Isolation and PCR amplification of genomic DNA from plant parasitic nematodes.* M.Sc. Project Report submitted to Acharya Nagarjuna University, Guntur, Andhra Pradesh.

b. Amina Shehrain, 2005. Designing of species specific primers for plant parasitic nematodes Radopholus and Pratylenchus. M.Sc. Project Report. Submitted to Bharathiar University, Coimbatore, Tamil Nadu.

c. Asha K.V. 2006. "Amplification of ribosomal genes in *Radopholus similis* using universal and species-specific primers". M.Sc. Project Report submitted to Bharatidasan University, Trichy, Tamil Nadu.

8235 Technical Bulletins / Extension Folders

a. Sarma, Y.R., Anandaraj, M. and Ramana, K.V. 1991. Tips on control of *Phytophthora* foot rot and slow decline disease of black pepper (Extension folder), National Research Centre for Spices, Calicut.

b. Ramana, K.V., Mohandas, C. and Santhosh J. Eapen, 1994. Plant parasitic nematodes and slow decline disease black pepper. National Research Centre for Spices. Calicut, pp. 14.

8236 Seminars, conferences and workshops (relevant to the project) in which the scientists have participated. (List abstracts forwarded)

a. National Seminar on Biological Control in Plantation Crops. Rubber Research Institute of India. Kottayam. 27 – 28 July, 1991.

b. Group meeting of Research Workers of All India Coordinated Research

Project on Spices, Trivandrum, 26-28 July, 1991.

c. Regional Workshop on Planning Agricultural Extension Training to Broad Base Extension. ICAR, Research Complex. Shillong. 25-27 November, 1991.

d. National Seminar on Black Pepper and Cardamom, 17-18 May 1992. Calicut, Kerala.

e. Second pepper (*Piper nigrum* L.) conference: current status and future prospects, 8-9 December, 1992. Kuching, Sarawak, Malaysia.

f. National Group Meeting of Research Workers of All India Coordinated Research Project on Spices. Kerala Agricultural University, Trichur, 26-28 July, 1993.

g. All India Coordinated Research Project on plant parasitic nematodes with integrated approach for their control. C.C.S. Haryana Agricultural University, Hissar, 3-5 August, 1993.

h. National Symposium on Recent Advances in Integrated Nematode Management of Agricultural Crops. C.C.S. Haryana Agricultural University, Hissar, 6-7 August, 1993.

i. National Seminar on Disease of Spices. Indian Society for Spices. Calicut, 7-8 April, 1994.

j. International Symposium on Plantation Crops (PLACROSYM XI), National Research Centre for Spices, Calicut, 30th November – 3rd December, 1994.

k. National group meeting on *Phytophthora* disease of horticultural crops. National Research Centre for Spices, Calicut. 21 – 23, September, 1994.

l. All Indian Coordinated Research Project on plant parasitic nematodes with integrated approach for their control. IARI, New Delhi, 22-23 March, 1995.

m. National Symposium on nematode problems of India. An appraisal of the nematode management with eco-friendly approaches and bio components. IARI, New Delhi, 24-26 March, 1995.

n. National Seminar on Biotechnology of Spices and Aromatic Plants (BIOSAAP), Calicut, Kerala, 24-25 April 1996.

o. Training on 'Computer Applications to Biological Research', CPMB, TNAU, Coimbatore, Tamil Nadu, 28-30 January 1997.

p. Summer school on 'Problems and Progress of Nematology During the Past one Decade' at IARI, New Delhi from 1-30 August 1997.

q. Tenth Biennial Group Meeting of All India Coordinated Research Project on Plant Parasitic Nematodes with Integrated Approach for their Control. Univ. of Agri. Sci. Bangalore. 23-26 September 1997.

r. Symposium on Economically Important Diseases of Crop Plants. Indian Phytopathological Society (South Zone). Univ. of Agril. Sci. Bangalore. 18-20 December 1997.

s. Group meeting of Nematologists of Horticultural Crops. C.P.C.R.I. Regional Station, Kayamkulam, Kerala. 16-18 January 1998.

t. Third International Symposium of Afro-Asian Society of Nematologists, Coimbatore, Tamil Nadu, 16-19 April 1998.

u. Golden Jubilee National Symposium on Spices, Medicinal and Aromatic Plants, 10-12 August 1998, Calicut

v. National Symposium on Rational Approaches in Nematode Management for Sustainable Agriculture, 23-25 November 1998, Anand, Gujarat.

w. PLACROSYM XIII, 15-18 December 1998, Coimbatore, Tamil Nadu.

x. XV Workshop of the All India Coordinated Research Projects on Spices. 18-21 November 1999. Calicut, Kerala

y. National Seminar on Nematological Research in India. 17 December 1999. C S Azad University of Agriculture and Technology, Kanpur

z. Centennial Conference on Spices, Medicinal and Aromatic Plants, 20-23 September 2000, Calicut.

aa. National Nematology Symposium on Integrated Nematode Management. Nov. 23-24, 2000. Orissa University of Agriculture & Technology, Bhubaneswar, Orissa.

bb. Fourth Management Development Programme in Agricultural Research. Nov. 30 – Dec. 6, 2000. National Academy of Agricultural Research Management, Hyderabad.

cc. National Congress on Centenary of Nematology in India. December 5-7, 2001, Indian Agricultural Research Institute, New Delhi.

dd. Indian Phytopathological Society South Zone Meeting, December 10-12, 2001, Indian Institute of Spices Research, Calicut.

ee. 'Nema 100 Kerala', February 21-22, 2002. Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala

ff. National Seminar on Strategies for Increasing Production and Export of Spices, 24-26 October 2002. Indian Society for Spices, Kozhikode.

gg. National Nematology Symposium, 17-19 November 2004, University of Agricultural Sciences, Bangalore.

hh. Meeting of the State Level Quality Control Team for monitoring the production of quality rooted pepper cuttings, 18 May 2005, Directorate of Agriculture, Trivandrum.

ii. Workshop on 'Biodiversity and Bioresources Utilization' organized by National Biodiversity Authority of India, Chennai, 21 January 2006, IISR, Calicut

jj. National Conference on Agro-biodiversity, 12-15 Feb. 2006, National Biodiversity Authority of India, Chennai.

824 Infrastructural facilities developed
(Details of field, laboratory, note books and final material and their location)

8241 Infrastructure developed

- Nikon research microscope with CCTV projection system
- Leica stereo microscope with image analyzing system
- Minor equipments like refrigerator, freezer, pH meter etc.

8242 Details of field, laboratory note books

Laboratory/field note books – 2 Nos. Available with Senior Scientist, Nematology

8243 Nematode resistant lines

All the promising lines are available in the germplasm repository of the Institute.

825 Comments / Suggestions of Project Leader regarding possible future line of work that may be taken up arising out of this Project

PART IV: PROJECT EXPENDITURE (Summary)

Year 1978-2005

		Estimated (Rs.)	Actual (Rs.)
830	**Total Recurring Expenditure**		
8301	Salaries: (Designation with pay scale)		
	i) Scientific	3,00,000	10,95,000
	ii) Technical	80,000	2,35,000
	iii) Supporting	50,000	1,74,000
	iv) Wages	-	53,000
	Sub-Total	4,30,000	15,57,000
8302	Consumables		
	i) Chemicals	70,000	98,000
	ii) Glassware	30,000	55,000
	iii) Others	20,000	46,000
	Sub-Total	1,20,000	1,94,000
8303	Travel	-	1,74,000
8304	Miscellaneous (other costs)	-	-
8305	Sub-Total (Recurring)	5,50,000	19,25,000
831	**Total Non – Recurring Expenditure** (Equipments and works)		
	i) Laminar flow unit	-	20,000
	ii) Research microscope	-	5,50,000
	iii) Refigerator	-	30,000
	iv) pH meter	-	10,000
	v) Stereomicroscope with image analyzing system	-	7,65,000
832	**Total** (830 and 831)	5,50,000	33,00,000

PART V: DECLARATION

This is to certify that the final report of the Project has been submitted in full consultation with the Project workers as per the approved objectives and technical programme and the relevant records, note-books, materials are available for the same.

Signature of the Project Investigator:

Co-Investigators 1. Nil

All the items of technical programme have been carried out and the relevant records are available in the Division. S Devasahayam

Signature & Comments of the Head of the Division/ Section

Signature & Comments of the Joint Director (Research)

Signature & Comments of the Director

ANNEXURE I

SURVEY

Survey for nematodes associated with ginger

Surveys were conducted in five districts of Kerala viz. Wyanad, Kozhikode, Ernakulam, Kottayam and Idukki Kerala during 1991-92 to identify the key plant parasitic nematodes associated with ginger crop. A total of 93 ginger fields were selected at random representing major growing areas in each district. A total of 209 samples each of soil and rhizomes were collected, processed and nematode population was estimated. Seven genera, *Meloidogyne, Rotylenchulus*, *Helicotylenchus, Xiphinema*, *Longidorus, Tylenchorhynchus* and *Criconemoides* were recorded.

Meloidogyne sp. was the most predominant species (100%, population range 51-561/ 100 cc soil) found in the rhizosphere of ginger. However, in Wyanad *Rotylenchulus reniformis* was the most predominant nematode in rhizosphere soils followed by root knot nematodes *Meloidogyne* sp. and *Helicotylenchus* sp. *Meloidogyne* was the only nematode genus isolated from rhizome samples.

Survey for nematodes associated with turmeric

A survey was conducted in collaboration with Regional Agricultural Research Station, Jagtial (APAU) in major turmeric growing areas of Adilabad, Nizamabad and Karimnagar districts of Andhra Pradesh. Fifty samples each of soil and rhizomes (Adilabad-10, Nizamabad-20, Karimnagar-20) were collected and processed at Plant Quarantine Regional Station (NBPGR), Hyderabad.

Results indicated that six genera of plant parasitic nematodes viz., *Meloidogyne, Rotylenchulus, Hoplolaimus, Criconemoides, Longidorus* and *Pratylenchus* were associated with turmeric crop. The most predominant nematode associated with turmeric was *Meloidogyne* (78%, population range 2127/100cc soil) followed by *Rotylenchulus* (64%, population range 23-215/100 cc soil). Other nematode genera recorded were *Hoplolaimus* (21%), *Criconemoides* (18%), *Longidorus* (16%) and *Pratylenchus* (14%). However, populations of these nematodes were low.

PATHOGENICITY

Pathogenicity of *M. incognita* on ginger

A pot culture experiment to assess the damage potential of *M. incognita* in ginger was conducted. There were five treatments replicated five times Seed rhizomes of cultivar 'Maran' @ 20 g/ pot were sown in earthen pots (12" dia) filled with sterilized soil mixture @ 10 kg/pot. One month after germination, nematodes were inoculated as per the treatments. Four months after inoculation the plants were harvested and observations recorded (Table 1).

Nematode inoculation caused considerable reduction in the growth parameters in ginger. Plants inoculated with the highest inoculum level i.e., 2.0 nematodes/g soil caused 16.04%, 21.87% and 16.97% reductions in plant height, number of tillers and weight of pseudostems, respectively though these reductions were not statistically significant. Root-knot index increased with increase in the inoculum level from 1.2 to 2.8.

Table 1. Effect of *Meloidogyne incognita* on growth of ginger

Treatment	Height (cm)	% Reduction	No. of tillers	% Reduction	Weight (g)	% Reduction	Root knot index
Control	62.4	-	6.4	-	61.5	-	0.0
0.5Nematodes/ g soil	61.3	1.79	5.6	12.50	65.9	-	1.2
1.0 "	56.3	9.83	5.6	12.50	58.9	4.19	2.2
1.5 "	55.7	10.75	4.8	25.00	52.4	14.79	2.8
2.0 "	52.4	16.04	5.0	21.87	51.1	16.97	2.8
CD 0.05%	N.S	-	N.S	-	N.S	-	-

Data are mean of five replications

Effect of *M. incognita* on growth and yield of ginger

A pot culture trial with different inoculum levels of *M. incognita* (0 - 200 J_2/100cc soil) was conducted to study the effect of *M. incognita* on growth and yield of ginger. Yield and growth characters were recorded after 9 months and they are presented in Table 2. The results indicate that *M. incognita* at Pi $\leq$ 0.2/100cc soil caused significant reduction in yield of ginger under test conditions.

Table 2. Growth and yield of ginger inoculated with *Meloidogyne incognita*

Treatment (J_2/100cc soil)	No. of tillers	Height (cm)	Shoot wt. (kg)	Root wt. (kg)	Rhizome wt. (kg)	Total biomas (kg)	Nematodes / root (Final)
0.0	60.62	83.62	0.724	0.350	1.439	2.5 13	-
0.2	76.50	78.62	0.828	0.361	1 .013	2.202	527.23
2.0	53.87	84.50	0.644	0.323	0.959	1.927	478.63
20.0	51.50	80.00	0.557	0.296	0.998	1.851	504.66
200.0	65.62	75.00	0.612	0.248	1.001	1.862	346.74
CD at 5%	NS	6.58	NS	0.077	0.327	0.498	NS

Data are mean of 8 replications

Damage potential of *M. incognita* to improved varieties of turmeric

A pot culture study was conducted to assess the damage potential of *M. incognita* to improved varieties of turmeric viz., Suvarna (PCT-8), Suguna (PCT-13), Sudarshana (PCT-14) and Alleppey. Earthern pots (20 cm dia.) were filled with 7 kg sterilized soil mixture. Seed rhizomes @ 30 g/pot of the test varieties (16 pots for each variety) were sown. One month after sowing eight plants in each variety were inoculated with freshly hatched second stage juveniles of *M. incognita* @ 3500/pot. Remaining eight plants in each variety were left as control. All the pots were arranged in net house and maintained. Seven months after sowing all the plants were harvested and observations on weight of leaf and pseudostem (dry), rhizomes (fresh), root (fresh), root-knot index and nematode population build up were recorded and data were analyzed statistically.

It was observed that nematode inoculation resulted in significant reduction in all the growth parameters recorded in all the four varieties tested (Table 3). Maximum reduction in dry weight of leaf and pseudostem was in Sudarshana (20.5%) followed by Suvarna. The reduction was the least in the variety Alleppey (11.2%). Similarly Alleppey recorded the minimum reduction in fresh weight of rhizome and root (11.9% and 9.6%, respectively). Maximum reduction in fresh rhizome weight was in Suvarna (18%) and fresh root weight in Suguna (21.2%). Among the four varieties of turmeric tested the damage caused by *M.incognita* infestation is the least in the variety Alleppey compared to Suvarna, Suguna, and Sudarshana.

Table 3. Effect of *M. incognita* on growth and yield of turmeric varieties

Variety/Treatment	Dry weight of leaf and pseudostem(g)	Weight of rhizomes(g)	Fresh weight of roots(g)
Suvarna (PCT-8)			
Control	17.68	235.26	53.08
Inoculated	14.07	192.45	46.30
Per cent reduction	20.40	18.20	12.70
Suguna (PCT-13)			
Control	15.45	281.43	28.90
Inoculated	13.12	237.57	22.75
Per cent reduction	15.00	15.50	21.20
Sudarshana (PCT-14)			
Control	15.00	303.36	23.07
Inoculated	11.92	258.90	19.26
Per cent reduction	20.50	15.70	16.50
Alleppey			
Control	37.45	388.51	98.33
Inoculated	33.25	342.16	88.81
Per cent reduction	11.20	11.90	9.60
CD (0.05)	1.41	17.54	3.48

INTERACTION STUDIES

Effect of *M. incognita, Pythium aphanidermatum* and *Ralstonia solanacearum* in the rhizome rot complex of ginger

A pot culture experiment to asses the role of *M. incognita* in rhizome rot complex of ginger was conducted. Seed rhizomes of ginger cultivar `Maran' @ 20 g/pot were sown in pots (12" dia) filled with 10 kg sterilized soil mixture. One month after germination the plants were inoculated with freshly hatched second stage juveniles of *M. incognita* @ 5,000 nematodes/pot as per the treatments. Twenty days after nematode inoculation the plants were inoculated with *Pythium* sp. and *Pseudomonas* sp. as per the treatments. Six replications were maintained per treatment. Incidence of the disease was monitored and the plants were harvested two months after final inoculation. The data are presented in Table 5.

Table 5. Effect of *M. incognita* in the disease incidence (Total of six replications)

Treatment	Healthy	Diseased	Total	* Onset of the disease	% of Disease
M. incognita (Mi) alone	29	0	29	-	Nil
Pythium (Py) alone	6	21	27	14	77.7
Pseudomonas (Ps) alone	0	26	26	5	100.0
Mi > Py	10	18	28	14	64.3
Mi > Ps	0	25	25	5	100.0
Py + Ps	0	25	25	6	100.0
Mi > Py + Ps	0	22	22	6	100.0
Control (No pathogens)	37	0	37	-	Nil

** Onset of disease in days from the date of fungal and bacterial inoculations.*
M.i = Meloidogyne incognita, Py = Pythium aphanidermatum, Ps = Pseudomonas solanacearum

Results indicated that symptoms of bacterial wilt started appearing from the 5th day after inoculation in plants inoculated with either *Pseudomonas* alone or in combination with *M incognita* and from 6th day onwards in plants inoculated with bacteria in combination with *Pythium* and *Pythium* and nematodes. Symptoms of *Pythium* infestation started appearing from 14th day after inoculation irrespective of nematode presence. Symptoms of bacterial wilt dominated in all the plants where bacteria and fungus were inoculated. In plants inoculated with fungus alone, 77.7% tillers showed infestation of the fungus while in plants inoculated with fungus and nematode 64.3% tillers showed fungal infestation two months after inoculation. Maximum number of tillers (37) were recorded in plants where no pathogens were inoculated followed by in plants inoculated with nematodes alone

(29). The results indicated that nematode inoculation prior to fungal/ bacterial inoculation neither enhanced disease incidence nor caused early incidence of the disease caused by either *Pseudomonas* or *Pythium* indicating no positive role of the nematode in the disease complex in ginger. However, the results have to be confirmed further.

The study was repeated using the ginger variety 'Himachal' and 10 treatments with four replications each. In treatments T8, T9 & T 10, the plants were inoculated with root knot nematodes 20 days prior to inoculating other pathogens and observation on the disease incidence were recorded one day after affecting all the treatments. The results of the study indicate that the plants inoculated with root-knot nematode alone did not show any symptoms of the disease as in the previous trial. Plants inoculated with *P. solanacearum* alone or in combination with nematode/fungus showed bacterial wilt symptoms (100%) on the 3rd day after bacterial inoculation except in treatment T10 where the incidence was 95.9%. This confirmed that the presence of root-knot nematode had no influence on the severity of bacterial wilt caused by *P. solanacearum* in ginger. However, prior inoculation of plants with root knot nematode resulted in increase in the severity of the rhizome rot disease and advanced the expression of disease symptoms. Plants inoculated with nematode and fungus simultaneously, only 16.1% tillers showed disease symptoms while in plants inoculated with nematodes 20 days prior to fungal inoculation, 46.5% tillers expressed disease symptoms within three days of fungal inoculation. All the tillers in plants inoculated with nematodes prior to fungal inoculation expressed disease symptoms by the 7th day whereas in plants inoculated with both the pathogens simultaneously, all the tillers expressed disease symptoms by 10th day after fungal inoculation. The study showed that root knot nematode predisposed ginger plants to fungal infection resulting in increased severity of the disease (Table 6).

Table 6. Role of *M. incognita* in rhizome rot and bacterial wilt in ginger

Treatment	Disease incidence (% tillers affected)		
	3 DAI*	7 DAI	10 DAI
T 1 Control (No Pathogen)	Nil	Nil	Nil
T2 *M. incognita* (Mi) alone	Nil	Nil	Nil
T3 *Pythium* (Py) alone	Nil	35.4	94.0
T4 *Pseudomonas* (Ps) alone	100.0	-	-
T5 Mi + Py (Simultaneously)	16.1	44.6	-
T6 Mi + Ps (Simultaneously)	100.0	-	100.0
T7 Mi + Py +Ps (Simultaneously)	100.0	-	-
T8 Mi > Py (20 days)	46.5	100.0	-
T9 Mi > Ps (20 days)	100.0	-	-
T10 Mi > Py + Ps (20 days)	95.9	100.0	-

*DAI = Days after inoculation

Effect of *M. incognita* and *Pythium aphanidermatum* in rhizome rot disease of ginger

To study the precise role of M. *incognita in* the rhizome rot of ginger a new trial has been carried in greenhouse. The final data on growth and yield are given in Table 7. The data showed that all treatments significantly reduced the total biomass as well as the yield. However, *Pythium* infections did not influence the nematode multiplication in any of the treatments. Combined inoculation of *Pythium aphanidermatum* and *M. incognita* was more damaging than either of them alone. This was more so when *Pythium* was inoculated 40 days after nematode inoculation.

Table 7. Effect of *Meloidogyne incognita* and *Pythium aphanidermatum* alone and in combination on growth, yield and nematode multiplication in ginger (Mean of five replications)

Treatment	No. of tillers	Root wt. (g)	Shoot wt. (g)	Rhizome wt. (g)	Total biomass (g)	Final nematode population	
						in root (per g)	in soil (per 100cc)
Check	9.25 a	127.5 a	105.50 a	270.00 a	506.00 a	-	-
MI alone	7.60 ab	118.0 a	91.00 ab	182.50 b	391.50 b	1806.34 a	123.71 a
Py alone	4.60 bc	35.0 b	57.50 b	181.25 b	273.75 bc	-	-
MI + Py	8.40 a	44.0 b	62.50 b	118.75 b	225.25 c	2218.29 a	13.84 a
MI>Py (20 d)	6.8 abc	71.0 ab	91.25 ab	195.00 b	357.25 bc	1607.68 a	40.72 a
MI > Py (40 d)	4.0 c	84.0 ab	66.25 b	167.50 b	317.75 bc	1038.01 a	38.51 a
Py>MI (40 d)	8.4 abc	45.0 b	65.00 b	127.50 b	237.50 c	1416.45 a	10.38 a

Means followed by a common letter in a column are not significantly different
MI- *Meloidogyne incognita, Py – Pythium aphanidermatum*

BIOLOGICAL CONTROL OF NEMATODES

Efficacy of Paecilomyces lilacinus in suppressing nematode infestations in black pepper

A pot culture study was conducted to assess efficacy of *Paecilomyces lilacinus* in suppressing nematode infestation in black pepper. Single noded rooted cuttings of Panniyur-1 were planted in pots with 6.5kg fumigated soil mixture. There were 10 treatments and each treatment was replicated five times (Table 4). After three months of planting, the fungus was incorporated into the root zone as per the treatments. Phorate 10G @ 3g/pot was applied at quarterly intervals starting from 3rd month after planting. *M. incognita* @ 2000 second stage juveniles/ plant and *R. similis* @ 100 nematodes/plant were introduced into the root zone 20 days after fungal inoculation as per the treatments. Twelve months after planting, all the plants were uprooted. Fresh weight of shoot, root, root knot index (RKI) and root lesion index (RLI) were recorded (Table 4).

Both the nematode species caused significant reduction in biomass production. *M. incognita* caused 18.1% and 31.6% reduction in fresh weight of shoot and root, respectively. Effect of *R. similis* inoculation was more severe, which resulted in 39.2% and 64.8% reduction in fresh weight of shoot and root. When both nematode species were inoculated reductions in these growth parameters were 61.2% and 74.5%.

Inoculation of the fungus and application of phorate improved growth of the vines and are on par. Fungus inoculation resulted in significant increase in growth parameters, even though the plants were inoculated with *M. incognita.* Loss in root mass due to *M. incognita* infestation was reduced from 31.6% to 21.1% in presence of the fungus. Similarly, the fungus inoculation resulted in increase in root mass production in plants inoculated with *R. similis.*

Root lesion index was maximum (3.6) in plants inoculated with *R. similis* alone followed by combined inoculation of two nematodes. When the fungus was incorporated, RLI was significantly reduced. Similar effects were also noticed in the treatment having fungus inoculated with *M. incognita*. RKI was brought down from 4.4 in plants inoculated with *M. incognita* alone to 2.4 in the presence of fungus.

The results indicated that though the fungus could not result in absolute control of the nematodes, it had significantly suppressed nematodes infestation and increased root mass production.

Table 4. Efficacy of *Paecilomyces lilacinus* in suppressing nematode infestations in black pepper

Treatment	Shoot wt. g (fresh)	Root wt. g (fresh)	RKI	RLI
Control	267.54 d	68.22 d	1.4f	1.4f
Phorate (Ph)	296.74 a (+10.9)	71.84 e (+5.3)	1.0g	1.0g
M. incognita (Mi)	219.06 g (-18.1)	46.64 g (-31.6)	4.4 a	1.0g
R. similis (Rs)	162.66 i (-39.2)	23.98 i (-64.8)	1.0 g	3.6a
P. lilacinus (Pl)	275.28 b (+2.9)	73.84 b (+8.2)	1.0 g	1.0g
Mi + Pl	230.72 g (-13.8)	82.72 a (+21.2)	2.4 c	1.0g
Rs + Pl	190.04 h (-28.9)	35.96 h (-47.3)	1.0 g	2.4c
Mi + Rs	103.80 g (-61.2)	17.40 j (-74.5)	2.8 b	3.4b
Mi + Rs + Pl	249.64 e (-6.7)	60.18 f (-11.8)	2.0 d	2.2 d
Mi + Rs + Ph	268.78 c (+0.5)	64.56 e(-5.4)	1.6 e	1.8 e
L.S.D. (P.05)	38.03	16.63	0.6	0.6

Figures in parentheses denote per cent reduction (-) / increase (+) over control.
Means followed by a common letter are not significantly different at 5% level.

Root lesion index was maximum (3.6) in plants inoculated with *R. similis* alone followed by combined inoculation of two nematodes. When fungus was incorporated, RLI was significantly reduced. Similar effects were also noticed in the treatment having fungus inoculated with *M. incognita*. RKI was brought down from 4.4 in plants inoculated with *M. incognita* alone to 2.4 inthe presence of the fungus.

The results indicated that though the fungus could not result in absolute control of the nematodes, it had significantly suppressed nematodes infestation and increased root mass production.

EFFECT OF MULCHING

Effect of mulching with *Chromolaena odorata* in suppressing root knot nematode infestation in black pepper

An observational trial in pot culture was undertaken to test the effect of mulching with *Chromolaena odorata* on suppression of root knot nematodes infestation in black pepper. Single noded rooted cuttings of Panniyur-1 were planted singly in earthen pots of 20 cm diameter containing 6.5kg fumigated soil mixture. Three months after planting, 30 plants were selected. Freshly hatched second stage juveniles of *M. incognita* @ 2, 000/plant were collected and were inoculated to 20 plants. Twenty days after inoculation, 0.5kg of *C. odorata* (fresh), cut into small bits was added as a mulch in 10 pots. The plants without nematode inoculation and mulch served as control. Four months after nematode inoculation, all the plants were depotted, root-knot index, fresh weight of shoot and root were recorded (Table 8).

Root knot index varied from 2.0 to 4.0 (average 3.3) in plants inoculated with nematodes and it ranged from 4.0 to 5.0 (average 4.3) in plants inoculated with nematode and mulched. Nematode inoculation caused significant reduction in fresh weight of shoot. However, there was increase in root weight (fresh) in plants inoculated with nematodes with or without mulch due to heavy root galling which contributed to increase in root weight.

The results showed that mulching pepper plants with *C. odoratum* did not help in reducing root knot nematode infestation and multiplication.

Table 8. Effect of *Chromolaena odoratum* mulching in suppressing root knot nematode infestation in black pepper.

Treatment	Shoot wt. g (fresh)	Root wt. g (fresh)	R. K.I.
Control	178.5	27.6	1.0
M. incognita	142.9 (-19.9)	32.5 (+17.7)	3.3
M. incognita + mulching	128.2 (-28.2)	37.7 (+36.6)	4.3
L.S.D. (P 0.05)	16.2	6.0	0.5

Figures in parenthesis denote % reduction (-) increase (+) over control.

Effect of different mulches on major nematodes of black pepper and ginger

A microplot study was conducted to evaluate the effect of various mulches on the incidence and multiplication of various nematodes infesting black pepper. For this, 40 black pepper vines were established in microplots containing fumigated soil mixture. The treatments were mulching with leaves of *Piper colubrinum*, *Strychnos nuxvomica*, application of vermicompost, and a check with normal recommended inputs.

The test plants were inoculated with nematodes and various treatments were imposed. The treatments were incorporated twice a year. Data on growth characters as well as nematode levels in soil and roots were monitored.

All the three organic materials improved the growth and yield of vines, *P. colubrinum* being the maximum (Table 9). However, none of them were able to provide complete protection against nematodes. Nematode populations, foliar yellowing and root rot were comparatively low in plants mulched with *S.nuxvomica* and *P.colubrinum* leaves.

Table 9. Effect of organic amendments on growth and yield of nematode-free and nematode-infested black pepper vines

Treatment	Canopy size[a]	Foliar yellowing Index[b]	Root rotting Index[b]	**Yield[c] (Kg)**
Check	2.62[d]	1.33	0.86	0.99
Nematode (N) alone	1.97	1.02	1.77	1.08
P. colubrinum (Pc)	2.90	0.47	1.12	1.70
S. nuxvomica (Sn)	2.96	1.08	0.72	1.28
Vermicompost (Vc)	2.75	1.49	2.52	1.15
Pc + N	1.22	1.02	0.98	1.22
Sn + N	1.45	0.71	1.09	0.98
Vc + N	1.58	1.87	2.14	0.94
LSD 0.05	1.12	N.S.	N.S.	N.S.

[a] & [b] Based on a scale of 1-5 and 0-5, respectively.
[c] Yield of green pepper/vine
[d] Data are weighted means based on the nematode population in individual plots.

STUDIES ON POPULATION VARIABILITY

Population variability in root knot nematodes associated with various spices

Fifty one populations *of* root-knot nematodes from black pepper, ginger, turmeric and other related species were collected from different areas in Kerala, Karnataka, Andhra Pradesh, Assam and Tamil Nadu. These populations were maintained on their respective hosts for studying their variability using morphological, biochemical and molecular tools.

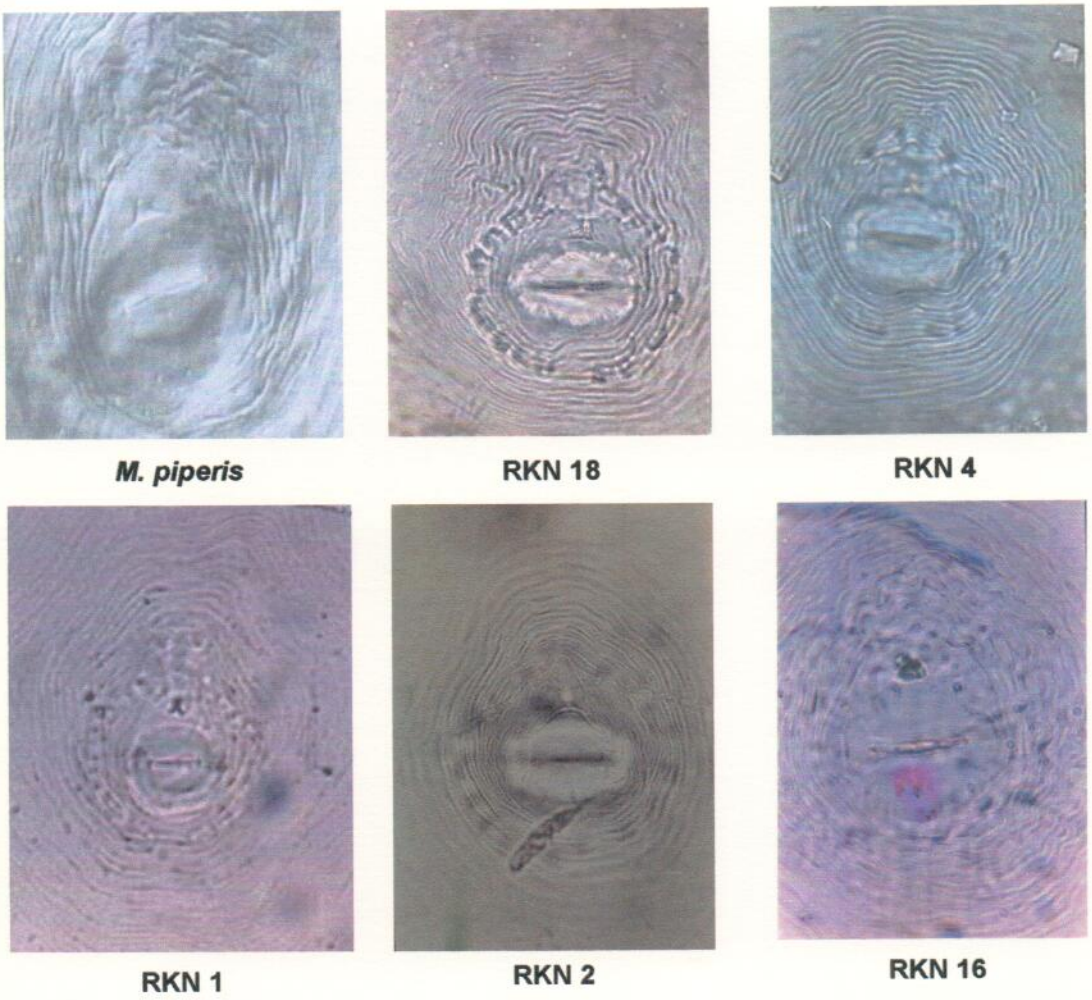


Fig. 1. Pereneal patterns of some selected root knot nematode populations from spices.

Perineal patterns of these populations were studied using Leica Qwin image analysis system which showed wide variability among these populations (Fig. 1). An isolate from Peruvannamuzhi, Kerala, infesting black pepper was identified as a new species, *M. piperis* with the help of Division of Nematology, IARI, New Delhi. Esterase and malate dehydrogenase patterns of the nematode populations were studied using native PAGE on Phast system. The patterns in some populations varied widely from the standard forms.

MOLECULAR CHARACTERIZATION

Genomic DNA isolation

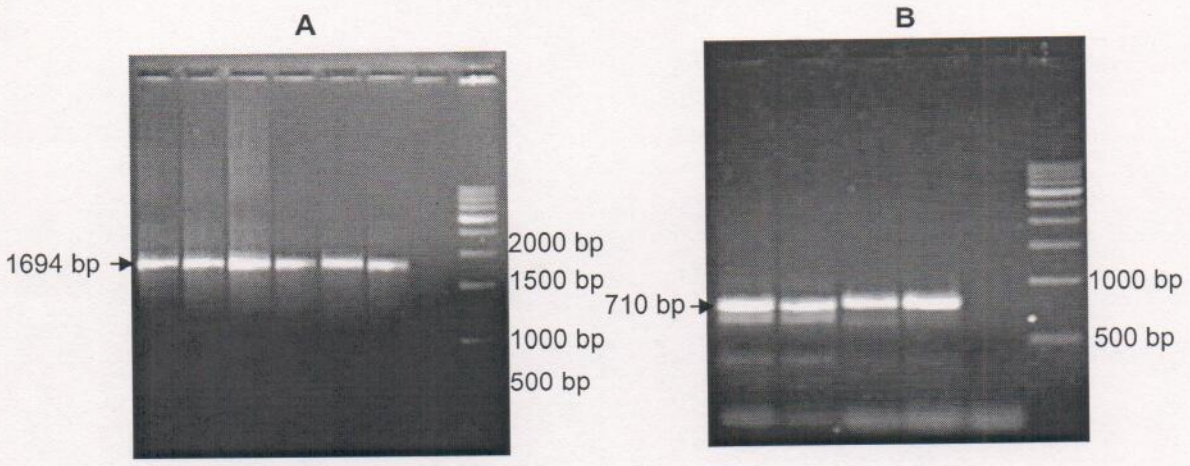


Fig. 2. Amplification of rDNA genes of *M.incognita* and *R.similis* using universal primers. A. 18S gene (Lane 1 to 3 – *M. incognita*, Lane 4 to 6 – *R. similis,* Lane 7 – negative control and Lane 8 – marker 500 bp ladder) B. D2/D3 expansion region (Lane 1 & 2 – *M. incognita,* Lane 3 & 4 - *R. similis,* Lane 5 – negative control and Lane 6 – marker 500 bp ladder)

Isolation of genomic DNA from root knot and burrowing nematode populations using two methods viz. phenol-chloroform-isoamyl alcohol method and sodium hydroxide digestion method was standardized. High quality DNA was isolated from these nematodes using phenol-chloroform-isoamyl alcohol method. Amplification of genomic DNA from a single nematode was also standardized using sodium hydroxide digestion method. The 18S and D2-D3 expansion region of rDNA was amplified using universal primers to get amplicons of 1694 bp and 710 bp, respectively (Table 10 and Fig. 2 a & b). The method needs more refinement to be used as reliable technique to be adopted for nematode biodiversity studies.

Table 10. Universal primers used for the amplification of rDNA genes of nematodes

Primer	Direction	Sequence
18S	Forward Primer	5'-GCTTGTCTCAAAGATTAAGCCATG-3'
	Reverse Primer	5'-TGATCCWKCYGCAGGTTCAC-3'
D2\D3 region of 28 S	Forward Primer	5-'ACAAGTACCGTGAGGGAAAGTTG-3'
	Reverse Primer	5'-'TCGGAAGGAACCAGCTACTA-3'

Primer designing

The rDNA species specific primers were designed using Bioinformatics tools for *R. similis* and *Pratylenchus* species. Nucleotide sequences of different strains of R. similis and Pratylenchus spp. were retrieved from GenBank. Multiple alignments of these nucleotide sequences were performed by using CLUSTAL X. The IUB weight matrix was kept default and the gap opening and extension parameters were excluded by setting null. Thermodynamic stability (melting temperature), GC% and self-complementarity (primer dimers) were calculated by using Oligo nucleotide properties (Calculator version 3.07 URL: http:// www. basic.northwestern.edu/ biotools/oligocalc.html). The chosen primers were then subjected for secondary structure (stem and loop) prediction by using MFOLD15 (URL: http:// www. bioinfo.rpi.edu/applications/mfold). The species-specific primers obtained are listed in Table 11.

Table 11. Species specific primers designed for *Radopholus* and *Pratylenchus* species

Species	Primer
Radopholus similis	5'-GAT TCC GTC CTT TGG TGG GCA-3' 3'-GGAGACCGTGCGGACCAAG-5'
Pratylenchus penetrans	5`-GGCAGGTAACAACGAGTCT-`3 3`--CCATGCAAGAACATTCGAGCG-5`
P. zeae	5`-CACGGTGCGTTCACCGACTAC-`3 3`- AGTCTTTCGCCCCTATACCC-`5
P. pratensis	5`-GAGACAGGTCGCTACCTTCTCGC-`3 3`-GCTACGTCAGCACAGATCCA-5`
P. loosi	5`- CGCTCAGTAACCCGCAAGTTTTGGG-`3 3`- TTCACCATCTTTCGGGTCTC-`5

PCR amplification

Amplification was performed with specific primer designed for *R. similis* in 10µl of reaction containing 1X buffer (10mM Tris pH 9, 50mM KCl, 0.01% gelatin), 200µm dNTP's mix, 1.5mM $MgCl_2$, 10µg BSA, 2pM each primer, 0.5 units of Taq DNA polymerase and template DNA at different concentration viz. 1ng, 10ng and 100 ng. DNA isolated from another nematode, *Meloidogyne incognita* was kept as a check. The thermocycling conditions consisted of an initial denaturation at 94^0C for 2 min, 35 amplification cycles of 94^0C for 30 sec, 65^0C for 1 min, 72^0C for 1 min and a final polymerization step of 72^0C for 10 min with Eppendorf master thermal cycler. The PCR product was separated on a 1% Tris Acetate EDTA buffered agarose gel. The above mentioned primer set amplified partially ITS 1 and 5.8 S region of ribosomal gene of *R. similis*. A product of 398 bp was

obtained when *R. similis* genomic DNA was amplified using the specific primers (Fig.3). No specific amplification was obtained for the genomic DNA of *M. incognita*. The result clearly proves the usefulness of these primers in detecting *R.similis* specifically. These primers were used for direct detection of *R. similis* from infested black pepper root tissues using PCR.

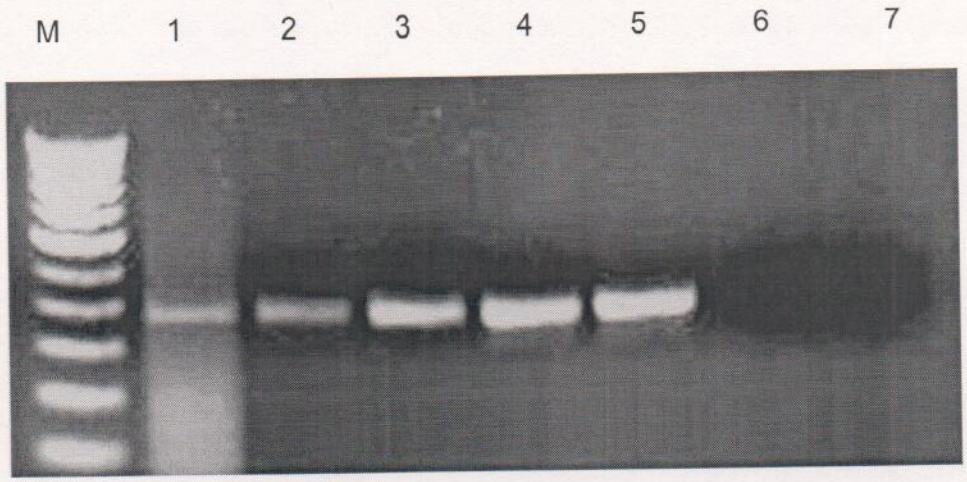


Fig. 3. Amplification of *R.similis* genomic DNA using species specific primer. M, marker 100 bp ladder; Lane 1 *R.similis* single nematode after re PCR; Lanes 2-4 *R.similis* genomic DNA in different concentrations (1ng,10ng and 100ng respectively); Lane 5 genomic DNA from *R.similis* infected root; Lane 6 *M.incognita* genomic DNA; Lane 7 genomic DNA from *M.incognita* infected roots.

Cloning and sequencing

The 398 bp product from 1% agarose gel was cut using a sterile scalpel and the DNA was eluted using gel extraction kit (Bangalore Genei). The concentration of the eluted product was quantified using Eppendorf biophotometer. This product was then ligated into Genei T vector (Genei instant cloning kit) according to manufacturer's instructions. The ligated product was then transformed into *E.coli* DH 5α cells by the standard heat shock transformation method. The recombinants were initially identified by blue / white selection. The white colonies were later screened by PCR using the specific and universal primers. The 398 bp insert was successfully released by restriction using *Nco I*. The clone was then sequenced at Bangalore Genei. The sequenced product was then compared with other sequences available in non redundant databases using BLAST programme.

Partial sequence of ITS 1 and 5.8 S regions of *R. similis* isolate showed 99% similarity with other *R. similis* isolates reported worldwide after pair wise comparison of nucleotides (Fig.4). There was only one nucleotide difference in ITS 1 region, where at the position

194, adenine is replaced by thymine. This is the first report of rDNA sequence from an Indian population of *R. similis*. The sequence was deposited in the nucleotide sequence database of EMBL (AM286692).

```
1    gaaccaggcg tgccagagga agtgactcca atggcgcaat gtgcattcga atgtttagtg
61   ttcaaaggtt ctgcagttca cactagatat cgcagctggc tgcgttcttc atcgacctac
121  gagccgagtg atccaccgat aaggctagaa ttcttgtttg ccttgaatgg catttgaaaa
181  agtttattgg aataaaatgg ttgttggcgg cgcttggtct gcgctcatca agtcttaagc
241  tgccaaaggc gatgacagcc ctaccgagtc gcatcatcgc agccacggac atggactaca
301  actgctccac gactcacaga cgccaaccca gccgttcgcg tcacatttga tgggtttcgc
361  caatgcctga ggggcactgc ccaccaaagg acggaatc
```

Fig.4. Partial sequence of ITS 1 and 5.8 S regions of Indian *R. similis* isolate from black pepper.

ANNEXURE II

LITERATURE CITED

Byrd, D.W. Jr., Nusbaum, C.J. and Barker, K.R. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15: 142-143.

Chen, CM., Li, H.Y. and Lii, O.Y. 1986. [The study on root-knot nematodes of common turmeric (*Curcuma domestica* Valet.)]. *Herald of Agricultural Sciences* 1: 16-22.

Cook, R. and Evans, K. 1987. Resistance and Tolerance. In *Principles and Practice of Nematode Control in Crops*. Edited by R.H. Brown and B.R. Kerry. Academic Press.

Coolen, W.A. and D'Herde, C.J. 1972. *A Method for the Quantitative Extraction of Nematodes from Plant Tissue*. Ghent State Agriculture Research Centre, Merelbeke, Belgium, 77pp.

Eapen, S.J. 1990. A methodology for evaluation of resistance in cardamom to root-knot nematodes. *Indian Journal of Nematology* 20: 197-202.

Eapen, S.J. 1995. *Investigations on Plant Parasitic Nematodes Associated with Cardamom – Final Report.* Indian Institute of Spices Research, Calicut, India. 39pp.

Gnanapragasam, N.C. 1989. Varietal response of pepper to infestation by the burrwing nematode, *Radopholus similis*. *Sri Lanka Journal of Tea Science* 58: 5-8.

Gunasekharan, C.R., Vadivelu, S. and Jayaraj, S. 1987. Experiments on nematodes of turmeric – a review. *Proceedings of the Third Group Discussions on the Nematological Problems of Plantation Crops, 29-30 October 1987,* Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India, pp. 45-46.

Hegde, L., Krishnappa, K. and Bojappa, K.M. 1993. Reaction of different cultivars of cardamom (*Elettaria cardamomum* Maton) to root knot nematode (*Meloidogyne incognita*). *Indian Journal of Nematology* 23: 127-128.

Holbrook, C.C., Knauft, D.A. and Dickson, D.W. 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria*. *Plant Disease* 57: 957 – 958.

Hussey, R.S. and Janssen, G.J.W. 2002. Root-knot nematodes: *Meloidogyne* spp. In: Plant Resistance to Parasitic Nematodes (Eds. J. L. Starr et al.), pp. 43 - 70. CABI Publishing, UK.

Jacob, A. and Kuriyan, J. 1979. Screening of pepper varieties for resistance against root-knot nematode (*Meloidogyne incognita*). *Agricultural Research Journal of Kerala* 17: 90.

Koshy, P.K. and Sundararaju, P. 1979. Response of seven black pepper cultivars to *Meloidogyne incognita*. *Nematologia Mediterranea* 7: 123-125.

Mani, A., Naidu, P.H. and Madhavachari, S. 1987. Occurrence and control of *Meloidogyne incognita* on turmeric in Andhra Pradesh. *International Nematology Network Newsletter* 4: 13-18.

O'Bannon, J.H. and Taylor, A.L. 1968. Migratory endoparasitic nematodes reared on carrot discs. *Phytopathology* 58: 385.

Paulus, A.D., Eng, L., Teo, C.H. and Sim, S.L. 1993. Screening of black pepper genotypes and *Piper* spp. for resistance to root-knot nematode. In: *The Black Pepper Industry –*

Problems and Prospects. University Pertanian Malaysia Bintulu Campus, Sarawak, Malaysia, pp. 132-139.

Ramana, K.V. 1992. *Role of Nematodes in the Incidence of Slow Decline (Slow Wilt Disease) of Black Pepper and Screening Pepper Germplasm Against Nematodes – Final Report.* National Research Centre for Spices, Calicut, India.

Ramana, K.V. and Mohandas, C. 1986. Reaction of black pepper germplasm to root-knot nematode, *Meloidogyne incognita*. *Indian Journal of Nematology* 16: 138-139.

Ramana, K.V. and Mohandas, C. 1989. Techniques for screening black pepper germplasm to *Radopholus similis* and *Meloidogyne incognita*. *Indian Journal of Nematology* 19: 144-149.

Ramana, K.V., Mohandas, C. and Ravindran, P.N. 1987. Reaction of black pepper germplasm to the burrowing nematode (*Radopholus similis*). *Journal of Plantation Crops* 15: 65-66.

Santhosh J. Eapen, Ramana, K.V., Sasikumar, B. and Johnson K. George. 1998. Resistance to Meloidogyne incognita in ginger and turmeric germplasms. In: Proceedings of the National Symposium on Rational Approaches in Nematode Management for Sustainable Agriculture, pp. 106-109. (Eds.) S.C. Dhawan & K.K. Kaushal, Nematological Society of India, New Delhi, India.

Starr, J.L., Bridge, J. and Cook, R. 2002. Resistance to plant parasitic nematodes: History, current use and future potential. In: Plant Resistance to Parasitic Nematodes (Eds. J. L. Starr et al.), pp. 1- 22. CABI Publishing, UK.

Venkitesan, T.S. and Setty, K.G.H. 1978. Reaction of 27 black pepper cultivars and wild forms to the burrowing nematode, *Radopholus similis* (Cobb) Thorne. *Journal of Plantation Crops* 6: 81-83.

ANNEXURE III

RESEARCH PUBLICATIONS

Journal of Plantation Crops 19 (1) : 41–53 June, 1991

PATHOGENICITY OF *MELOIDOGYNE INCOGNITA* AND *RADOPHOLUS SIMILIS* ON BLACK PEPPER (*PIPER NIGRUM* L.)*

C. MOHANDAS[1] and K. V. RAMANA

National Research Centre for Spices, Kozhikode 673 012, Kerala, India

ABSTRACT

Root knot nematode, *Meloidogyne incognita* and the burrowing nematode, *Radopholus similis* are highly pathogenic to black pepper *(Piper nigrum* L.) and affected the growth and vigour of the vines resulting in significant reduction in yield. The damage caused by *R. similis* to the pepper vines was more severe. Pepper vines inoculated with *R. similis* exhibited severe foliar yellowing, defoliation and die back, typical symptoms associated with slow decline (slow wilt) of black pepper. The disease first appeared as mild foliar yellowing which later intensified with time leading to defoliation and die back. In general foliar yellowing and defoliation were low during July and high during April / May.

INTRODUCTION

Black pepper *(Piper nigrum* L.) the 'King of Spices' is grown mostly in Kerala and Karnataka states in India. Slow decline (Slow wilt/Pepper yellows) a major constraint in black pepper production attributed to plant parasitic nematodes is prevalent in all the pepper growing areas in India. As early as in 1906, Butler reported root knot nematode infestation in black pepper in Wynad, Kerala (Butler, 1906). Association of the burrowing nematode, *Radopholus similis* with black pepper in India was first reported from South western India (D'Souza et al., 1970). Among the fourteen genera of plant parasitic nematodes found in association with black pepper (Ramana and Mohandas, 1987), *Meloidogyne incognita* and *R. similis* are the two major endoparasitic nematodes infesting the crop in Kerala and Karnataka (Kumar, Viswanathan and D'Souza, 1971; Venkitesan, 1972; Jacob and Kurian, 1979; Anonymous, 1986; Ramana and Mohandas, 1987). Further, these two nematode species were implicated in the etiology of slow decline of black pepper in India (Nambiar and Sarma, 1977; Venkitesan and Setty, 1977; Ramana, Mohandas and Balakrishnan, 1987) and also in other pepper growing countries (Van der Vecht, 1950; Christie, 1957; Hubert, 1957; Ting, 1975; Ichinohe, 1976; Bridge, 1978; Mustika, 1978). Reduction in the growth of

* *Contribution No. 122, National Research Centre for Spices, Kozhikode*

1 *Present address : Central Tuber Crops Research Institute, Sreekariyam 695 017, Thiruvananthapuram, Kerala*

pepper vines due to *M. incognita* (Koshy et al., 1979; Jacob and Kurian, 1979; Ferraz and Sharma, 1979; Mohandas and Ramana, 1983; Mustika, 1984) and *R. similis* (Venkitesan, 1976; Venkitesan and Setty, 1977; Mustika, 1984) were reported. However, in all these studies the actual loss in the yield was neither estimated nor the symptoms of slow decline were reproduced since these studies were conducted in pot culture under greenhouse conditions on young vines and terminated before the vines attained maturity. To bridge the gap, pathogenicity tests were conducted under simulated field conditions on grown up vines.

MATERIALS AND METHODS

The experiments were conducted in an area of 100 m length × 70 m wide, fenced with barbed wire. Cement tubs (1 m diameter and 1 m height) with two holes (2 cm dia) at bottom at equidistance, were burried leaving top 30 cm of the tubs above the ground level at 3 m apart. Trenches of 50 cm wide and 1 m deep were dug lengthwise in the middle of the interspaces. Side channels perpendicular to the main channel were also dug on both sides of the tubs (micro plots) for effective drainage. Glass pieces (10 × 10cm) were placed near the holes inside the tubs. A layer of (10 cm thick) gravel and sand was spread in the bottom of the tubs and then filled with soil mixture(forest soil 3: sand 1: cowdung 1) upto 15 cm below the rim of the tubs. The whole area under the experiment and the soil mixture in the tubs were fumigated with methyl bromide. Three weeks after fumigation *Glyricidia sepium* stumps (1 m length) were planted in south west direction near each tub for trailing pepper vines. Saplings of *Acacia auriculiformis* were planted in the interspaces and all around the experimental area for providing shade and natural environment for growing pepper vines. The plot was divided into two equal halves consisting of 70 micro plots each. One half was further divided into two consisting of 35 micro plots each (block A & B). In block A, experiment on pathogenicity of *M. incognita* and in block B, experiment on pathogenicity of *R. similis* were conducted. In the remaining half consisting of 70 micro plots (block C), experiment on pathogenicity of *M. incognita* and *R. similis* in combination was laid out. Rooted cuttings of black pepper hybrid 'Panniyur–1' raised in fumigated soil mixture were used for planting. At the time of planting the rooted cuttings in the micro plots, three PVC pipes (20 cm long; 2 cm dia) were burried around the cuttings to reach different depths *viz.*, 5 cm, 10 cm and 15 cm in the root zone to facilitate uniform distribution of inoculum in the root zone. Freshly hatched second stage juveniles of *M. incognita* from the egg masses collected from black pepper roots and *R. similis* population multiplied on carrot discs were used for inoculation. Regular watering and manuring were done as per the recommended schedule.

Pathogenicity of *M. incognita*

Rooted cuttings of 'Panniyur–1' were planted singly in the micro plots during July, 1983. Two months after planting 25 plants with uniform growth and vigour were selected and inoculated with *M. incognita* @ 100, 1,000, 10,000 and 1,00,000 second stage juveniles per

vine in five replications along with uninoculated control. The treatments were distributed at random.

Pathogenicity of *R. similis*

Rooted cuttings of 'Panniyur–1' were planted in the micro plots during June 1984. One year after planting the vines were inoculated with different levels of *R. similis viz.*, 10, 100, 1,000 and 10,000 per vine in seven replications at random. Seven vines left uninoculated served as controls.

Pathogenicity of *R. similis* and *M. incognita* in combination

Rooted cuttings of 'Panniyur–1' were planted in the micro plots during June 1984. One year after planting 66 vines with uniform growth and vigour were selected and inoculated with nematodes in six replications as follows :

1) Uninoculated – control
2) *M. incognita* 500
3) *M. incognita* 1000
4) *R. similis* 500
5) *R. similis* 1000
6) *M. incognita* 500 + *R. similis* 500 (simultaneously)
7) *M. incognita* 500 + *R. similis* 500 (20 days after)
8) *R. similis* 500 + *M. incognita* 500 (20 days after)
9) *M. incognita* 1000 + *R. similis* 1000 (simultaneously)
10) *M. incognita* 1000 + *R. similis* 1000 (20 days after)
11) *R. similis* 1000 + *M. incognita* 1000 (20 days after)

All the treatments were distributed at random.

In all the experiments foliar yellowing index (F.Y.I.) on 1–4 scale (1 = No leaves showing yellowing; 2 = upto 20 per cent leaves showing yellowing; 3 = > 20 per cent and upto 60 per cent leaves showing yellowing and 4 = > 60 per cent leaves showing yellowing) and defoliation index (D.F.I.) on 1–4 scale (1 = < 10 per cent defoliation; 2 = > 10 per cent and upto 30 per cent defoliation; 3 = > 30 per cent and upto 60 per cent defoliation, and 4 = > 60 per cent defoliation) were recorded at quarterly intervals. At the time of concluding the experiments (December, 1987) individual vines were removed from the micro plots alongwith the root system by flushing out the soil with gentle flow of water. Maximum care was taken to extract all the roots from the micro plots. The following observations were also recorded. (1) Height of the vine, (2) Number of primary shoots, (3) Fresh and dry weights of shoot, leaf and root, (4) Root knot index (R.K.I.) on 0–5 scale (Taylor and Sasser, 1978) and Root lesion index (R.L.I.) on 1–5 scale (Ramana, Mohandas and Ravindran, 1987). Nematode population in soil (100 cc) and in roots (one gram) were estimated.

RESULTS AND DISCUSSION

Pathogenicity of *M. incognita*

Foliar yellowing and defoliation indices recorded from October 1983 onwards at quarterly intervals are given in Table I. The results show that *M. incognita* caused foliar yellowing and

Table I. *Foliar yellowing (FYI) and defoliation (DFI) indices of black pepper vines inoculated with* Meloidogyne incognita

Inoculum level	Year / Month																
	1983	1984				1985				1986				1987			
	OCT	JAN	APR	JUL	OCT	JAN	APR	JUL	OCT	JAN	APR	JUL	OCT	JAN	APR	JUL	OCT
Uninoculated control																	
FYI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DFI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
100 Nematodes																	
FYI	1.20	1.20	1.20	1.00	1.00	1.20	1.60	1.00	1.20	1.40	1.60	1.40	1.40	1.40	1.40	1.80	1.40
DFI	1.00	1.00	1.00	1.00	1.00	1.00	1.20	1.00	1.00	1.00	1.00	1.40	1.20	1.00	1.20	1.00	1.20
1000 Nematodes																	
FYI	1.00	1.00	1.40	1.00	1.00	1.60	1.60	1.20	1.40	1.40	1.60	1.60	1.40	1.40	1.60	1.40	1.60
DFI	1.00	1.00	1.00	1.00	1.00	1.00	1.20	1.20	1.00	1.20	1.40	2.00	1.20	1.20	1.80	1.00	1.40
10,000 Nematodes																	
FYI	1.00	1.00	1.50	1.25	1.50	2.00	2.25	1.50	1.50	1.75	1.75	1.25	1.75	1.50	1.75	1.50	1.75
DFI	1.00	1.00	1.00	1.00	1.00	1.00	1.50	1.25	1.25	1.25	1.00	1.50	1.25	1.25	1.50	1.25	1.50
1,00,000 Nematodes																	
FYI	1.60	1.00	2.00	1.40	1.40	1.60	1.60	1.20	1.40	1.20	1.80	1.40	1.40	1.60	1.80	1.60	1.60
DFI	1.00	1.00	1.00	1.00	1.00	1.00	1.60	1.40	1.60	1.20	1.60	1.40	1.00	1.20	1.60	1.20	1.40

defoliation which varied with inoculum level and also during different periods. In general these indices were high in the vines inoculated with higher inoculum levels (10,000 and 1,00,000 nematodes) and were low in the vines inoculated with lower inoculum levels (100 and 1000 nematodes).

Data on the growth parameters and yield are given in Table II. There was a significant reduction in the height of the vines inoculated with 10,000 (20.34 per cent) and 1,00,000 (19.98 per cent) nematodes while it was not significant in the vines inoculated with 100 and 1000 nematodes. Similarly maximum reduction in the number of primary shoots was recorded in the vines inoculated with higher inoculum levels. However, there was no significant difference in the number of primary shoots between the vines inoculated with lower inoculum levels and uninoculated (control) vines. Dry weights of shoot, leaf and root were also affected by nematode inoculation and the reduction increased with increase in the inoculum level. Maximum reduction was in the vines inoculated with highest inoculum level (1,00,000 nematodes).

Winoto (1972) also reported significant reduction in the growth of black pepper var. Kuching due to inoculation with *M. incognita* and *M. javanica*. Significant reduction to the dry weight of shoot and root in black pepper cv. 'Singapura' was recorded when inoculated with 6000 juveniles of *M. incognita* at fifteen months after inoculation (Ferraz and Sharma, 1979). Similar effects on the growth of black pepper due to root knot nematode were reported (Koshy et al., 1979; Jacob and Kurian, 1979; Ferraz and Sharma, 1979; Lamberti, Rohini and Ekanayake, 1983; Mohandas and Ramana, 1983).

Table II. *Effect of* Meloidogyne incognita *on the growth and yield of black pepper*

Inoculum level	Height of the vine (cms)	No. of primary shoots	Dry weight (g)			Yield (g) dry berries (1987)
			Shoot	Leaf	Root	
Uninoculated control	334.20	26.80	1700.96	410.08	194.56	580.2
100 Nematodes	316.00 (5.44)	23.20 (13.43)	1493.16 (12.21)	399.90 (2.48)	215.58 —	505.8 (12.82)
1000 Nematodes	309.80 (7.30)	22.20 (17.16)	1226.44 (27.89)	321.44 (21.61)	166.26 (14.54)	509.6 (12.16)
10,000 Nematodes	226.20 (20.34)	16.60 (38.05)	963.40 (43.36)	289.18 (29.48)	148.82 (23.50)	361.2 (37.74)
1,00,000 Nematodes	267.40 (19.98)	16.40 (38.80)	811.98 (52.26)	277.38 (32.35)	130.74 (32.80)	308.0 (46.91)
C. D. at 5%	53.13	6.13	299.18	67.68	47.15	47.97

Figures in parentheses are percentage reduction over uninoculated control

M. incognita infestation affected the yield of black pepper. The reduction in the yield was significant in the vines inoculated with 1,00,000 nematodes (46.91 per cent) and 10,000 nematodes (37.74 per cent). The reduction was about 12 per cent in the vines inoculated with lower inoculum levels and it was significant compared to the yield of uninoculated vines.

Root knot index and nematode population in soil and root increased with increase in the inoculum level and were maximum in the vines inoculated with 1,00,000 nematodes (Table III).

Pathogenicity of *R. similis*

Foliar yellowing and defoliation indices recorded at quarterly intervals are given in Table IV. These indices increased with increase in the inoculum level and also intensified with time. Vines inoculated with higher inoculum levels (1,000 and 10,000 nematodes) exhibited foliar yellowing during the first quarter after inoculation which became severe subsequently with the F.Y.I. more than 3 during 1987. Similar trend was also noticed with regard to defoliation. However, there was a general reduction in these indices during July.

Table III. *Root knot index (RKI) and nematode population in black pepper vines inoculated with* Meloidogyne incognita

Inoculum level	R.K.I.	Nematode population	
		Soil (100 cc)	Root (1 g)
Uninoculated control	0.00	Nil	Nil
100 Nematodes	1.60	54.8	760
1000 Nematodes	2.80	169.8	1950
10,000 Nematodes	4.25	237.8	3275
1,00,000 Nematodes	4.60	295.6	3640

Data on the growth characteristics and yield are given in Table V. Height of the vines was significantly reduced when inoculated with 1,000 (19.81 per cent) and 10,000 (19.63 per cent) nematodes which were on par. More than 40 per cent reduction in the number of primary shoots was recorded in the vines inoculated with 100 nematodes and more. Similarly the nematode caused significant reduction in the dry weights of shoot, leaf and root. Even the lowest inoculum of 10 nematodes caused 24.74 per cent, 25.34 per cent and 34.47 per cent reduction in the dry weights of shoot, leaf and root respectively. More than 75 per cent reduction in the dry weight of leaf was recorded in the vines inoculated with higher inoculum levels indicating the severity of defoliation caused by nematode infestation. Venkitesan (1976) under artificial inoculation with *R. similis* in pot culture found significant reduction in the shoot length and weight of black pepper rooted culture at 150 days after inoculation.

It is also evident from the results that *R. similis* caused severe damage to the roots. The reduction in the root mass was significant even in the vines inoculated with 10 nematodes (34.47 per cent). Maximum reduction of 81.81 per cent was recorded in the vines inoculated with 10,000 nematodes. The damage caused to the roots reflected on

Table IV. *Foliar yellowing index (FYI) and defoliation index (DFI) of black pepper vines inoculated with* Radopholus similis

Inoculum level	Year / Month								
	1985	1986				1987			
	OCT	JAN	APR	JUL	OCT	JAN	APR	JUL	OCT
Uninoculated control									
FYI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DFI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
10 Nematodes									
FYI	1.00	1.14	1.57	1.28	1.42	1.14	1.71	1.14	1.57
DFI	1.00	1.28	1.28	1.00	1.00	1.00	1.14	1.00	1.28
100 Nematodes									
FYI	1.42	1.71	1.85	1.28	2.42	2.28	2.57	1.71	2.71
DFI	1.14	1.14	1.28	1.14	1.14	1.85	2.28	1.00	2.14
1000 Nematodes									
FYI	2.41	2.14	2.71	1.71	2.71	3.14	3.42	3.00	3.42
DFI	1.28	1.42	1.85	1.42	2.14	2.85	3.14	2.85	3.14
10,000 Nematodes									
FYI	2.14	2.42	2.71	1.42	2.57	3.57	3.57	3.14	3.57
DFI	1.42	1.85	2.14	2.14	2.85	2.71	3.28	3.14	3.28

Table V. *Effect of* Radopholus similis *on the growth and yield of black pepper*

Inoculum level	Height of the vine (cm)	No. of primary shoots	Dry weight (g)			Yield (g) dry berries (1987)
			Shoot	Leaf	Root	
Uninoculated control	329.57	31.28	2180.31	462.34	235.05	465.71
10 Nematodes	328.85 (0.21)	27.14 (13.23)	1640.72 (24.74)	345.14 (25.34)	154.01 (34.47)	464.28 (0.30)
100 Nematodes	291.28 (11.61)	17.42 (44.30)	1245.22 (42.88)	204.50 (55.76)	90.48 (60.50)	330.71 (28.98)
1,000 Nematodes	264.28 (19.81)	16.14 (48.40)	975.21 (55.27)	89.05 (80.73)	70.88 (69.84)	232.14 (50.15)
10,000 Nematodes	264.85 (19.63)	13.57 (56.61)	854.81 (60.83)	105.74 (77.12)	42.57 (81.88)	188.71 (59.47)
C.D. at 5%	45.65	6.40	371.90	69.11	31.43	82.76

Figures in parentheses are percentage reduction over uninoculated control

the growth and yield of the vines. Yield of black pepper was significantly reduced in the vines inoculated with 100 nematodes and more, the maximum reduction being 59.47 per cent with 10,000 nematodes followed by 50.15 per cent with 1,000 nematodes.

Root lesion index, nematode population in soil and root are given in Table VI. Root lesion index increased with the increase in the initial inoculum level and ranged from 1.57 to 3.85. The final nematode population did not differ much in the higher inoculum levels indicating that there was no linear increase in nematode build up with the increase in the inoculum level.

Pathogenicity of *M. incognita* and *R. similis* in combination

Data on foliar yellowing and defoliation indices (Table VII) showed that these indices were always high in the vines inoculated with *R. similis* alone or in combination with *M. incognita* compared to the indices of the vines inoculated with *M. incognita* alone. At the time of concluding the experiment the F.Y.I. was maximum (3.50) in the vines inoculated with both the nematodes simultaneously @ 1000 nematodes each. Vines inoculated with *R. similis* alone or in combination with *M. incognita* (1,000 nematodes each) had the F.Y.I. more than 3. The F.Y.I. was low in the vines inoculated with *M. incognita* alone. Mustika (1978) in pot culture studies observed a close relationship between the population levels of *M. incognita* and *R. similis* and the onset of the 'yellows' disease in Indonesia. Similarly, the vines inoculated with *R. similis* alone or in combination with *M. incognita* at both levels of inoculation showed higher D.F.I. compared to that in the vines inoculated with *M. incognita* alone.

Table VI. *Root lesion index (RLI) and nematode population in black pepper vines inoculated with* Radopholus similis

Inoculum level	R.L.I.	Nematode population	
		Soil (100 cc)	Root (1 g)
Uninoculated control	1.00	Nil	Nil
10 Nematodes	1.57	6.85	17.14
100 Nematodes	2.42	15.14	102.14
1000 Nematodes	3.71	36.28	163.57
10,000 Nematodes	3.85	33.85	209.28

In the case of growth characteristics and yield the effect of *R. similis* was more conspicuous (Table VIII). Height of the vine was reduced significantly when inoculated with *R. similis* @ 1000 alone or in combination with *M. incognita* @ 1000 (ranged from 11.99 per cent to 22.01 per cent). *M. incognita* 500 alone did not cause significant reduction in the height of the vine while it was significant when inoculated in combination with *R. similis*. Number of primary shoots were reduced significantly in the vines inoculated with *R. similis* at both the levels alone and in combination with *M. incognita*. Maximum reduction of 53.51 per cent was recorded in the vines inoculated with *R. similis* along with *M. incognita* @ 1000 nematodes each.

Dry weight of shoot was significantly reduced with *R. similis* inoculation. Even 500 nematodes caused a

significant reduction in the dry weight of shoot (43.66 per cent). *M. incognita* alone at 500 and 1000 nematodes caused significant reduction in the shoot dry weight over uninoculated controls. More than 60 per cent reduction was recorded in the vines inoculated with *R. similis* 500 and 1000 alone or in combination with *M. incognita*. Similarly *R. similis* inoculation caused significant reduction in the dry weight of leaves, maximum being 81.86 per cent in the vines inoculated with *R. similis* 1000. *M. incognita* alone at lower levels did not cause significant reduction in the leaf dry weight. There was no significant difference in the dry weight of root of the vines inoculated with *M. incognita* (both inoculum levels) and that of uninoculated vines. However, *R. similis* even at the lower inoculum level (500 nematodes) significantly affected the root growth (47.23 per cent reduction). The reduction was more than 70 per cent in combination with *M. incognita*. Mustika (1984) also reported significant reduction

Table VII. *Foliar yellowing index (FYI) and defoliation index (DFI) of black pepper vines inoculated with* Meloidogyne incognita *and* Radopholus similis *and in combinations*

Inoculum level		Year / Month								
		1985	1986				1987			
		OCT	JAN	APR	JUL	OCT	JAN	APR	JUL	OCT
Uninoculated control	FYI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	DFI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MI 500	FYI	1.16	1.50	1.33	1.00	1.00	1.00	1.16	1.00	1.16
	DFI	1.00	1.16	1.00	1.16	1.00	1.16	1.16	1.00	1.00
MI 1000	FYI	1.16	1.33	1.66	1.33	1.16	1.00	1.33	1.00	1.33
	DFI	1.00	1.16	1.00	1.16	1.00	1.33	2.00	1.50	1.66
RS 500	FYI	1.50	2.00	2.16	1.33	1.16	1.66	2.16	1.83	2.00
	DFI	1.50	1.83	1.66	1.83	1.33	2.00	2.83	2.50	3.33
RS 1000	FYI	2.00	2.50	2.50	1.83	2.50	3.16	3.83	3.16	3.33
	DFI	1.33	1.66	1.66	1.33	2.33	2.50	3.16	3.16	3.33
MI 500+RS 500 (S)	FYI	1.16	1.50	1.33	1.00	1.00	1.00	2.16	2.00	2.50
	DFI	1.16	1.33	1.16	1.33	1.00	1.50	2.50	2.16	2.16
MI 500+RS 500 (20 DA)	FYI	1.50	2.00	1.83	1.50	2.00	2.50	2.50	2.16	2.16
	DFI	1.16	1.16	1.00	1.16	1.50	2.00	2.50	2.00	2.16
RS 500+MI 500 (20 DA)	FYI	1.33	1.66	2.00	1.50	1.66	2.16	3.00	2.50	3.00
	DFI	1.16	1.16	1.00	1.50	1.16	2.00	2.66	3.16	3.16
MI 1000+RS 1000 (S)	FYI	1.83	2.16	2.33	1.83	1.83	3.00	3.33	2.83	3.50
	DFI	1.33	1.50	1.16	1.16	1.50	2.50	2.83	3.33	3.50
MI 1000+RS 1000 (20 DA)	FYI	2.16	2.16	2.33	1.33	2.00	2.33	3.00	2.66	3.16
	DFI	1.33	1.33	1.33	1.33	1.16	2.16	3.16	2.66	3.16
RS 1000+MI 1000 (20 DA)	FYI	2.00	2.33	2.16	1.33	1.50	2.16	3.00	2.33	3.16
	DFI	1.66	1.50	1.83	1.50	1.00	1.66	2.66	2.66	3.33

MI – *M. incognita;* RS – *R. similis;* S – Simultaneously; 20 DA – 20 days after first inoculation

in the growth of pepper plants inoculated with *M. incognita* and *R. similis* alone or together.

Yield of black pepper was severely affected by nematode inoculation. Maximum reduction (64.63 per cent) was in the vines inoculated with both the nematodes @ 1000 each followed by *R. similis* 1000 alone (61.06 per cent). The lower inoculum level of *R. similis* alone or in combination with *M. incognita* also caused significant reduction in the yield ranging from 38.46 to 56.35 per cent. However, *M. incognita* at both the levels did not affect the yield.

Root knot and root lesion indices of the vines inoculated with two levels of the nematodes alone and in combi-

Table VIII. *Effect of* Meloidogyne incognita *and* Radopholus similis *on the growth and yield of black pepper*

Inoculum level	Height of the vine (cm)	No. of primary shoots	Dry weight (g)			Yield (g) Dry berries (1987)
			Shoot	Leaf	Root	
Uninoculated control	346.00	26.11	1524.00	494.86	184.93	530.83
MI 500	339.50 (1.87)	24.00 (8.25)	1279.50 (16.05)	472.93 (4.43)	184.38 (0.29)	509.16 (4.08)
MI 1000	307.00 (11.27)	20.16 (22.93)	1139.06 (25.26)	380.01 (23.20)	149.23 (19.30)	489.16 (7.84)
RS 500	309.00 (10.69)	15.16 (42.04)	858.68 (43.66)	191.08 (61.38)	97.58 (47.23)	231.66 (56.35)
RS 1000	269.83 (22.01)	13.00 (50.30)	529.90 (65.23)	89.76 (81.86)	55.31 (70.09)	206.66 (61.06)
MI 500+RS 500 (S)	321.66 (7.03)	14.83 (43.31)	1063.10 (30.25)	208.21 (57.92)	89.73 (51.47)	293.33 (44.74)
MI 500+RS 500 (20 DA)	308.83 (10.74)	14.66 (43.96)	918.00 (39.77)	183.66 (62.88)	80.60 (56.41)	326.66 (38.46)
RS 500+MI 500 (20 DA)	316.50 (8.52)	16.83 (35.66)	939.51 (38.36)	188.56 (61.89)	85.51 (53.76)	247.50 (53.37)
MI 1000+RS 1000 (S)	284.33 (17.82)	12.16 (53.51)	419.55 (72.47)	122.24 (75.24)	53.75 (70.93)	187.75 (64.63)
MI 1000+RS 1000 (20 DA)	296.66 (14.26)	13.33 (49.04)	568.98 (62.67)	108.23 (78.12)	45.63 (75.32)	238.33 (55.10)
RS 1000+MI 1000 (20 DA)	304.50 (11.99)	13.00 (50.30)	568.05 (62.73)	157.95 (68.08)	51.81 (71.98)	220.83 (58.39)
C.D. at 5%	35.11	5.11	208.29	65.66	28.80	85.24

Figures in parentheses are percentage reduction over uninoculated control
MI – *M. incognita;* RS – *R. similis;* S – Simultaneously; 20 DA – 20 days after first inoculation

nations are given in Table IX. There was an increase in these indices with the increase in the inoculum level from 500 to 1000 nematodes when inoculated separately. In the vines inoculated with both the nematode species, the R. K. I. were less compared to the indices of the vines inoculated with *M. incognita* alone at both the inoculum levels. On the contrary, the R. L. I. were almost same in the vines inoculated with *R. similis* alone or in combination with *M. incognita* at both inoculum levels. Similar trend was observed in the case of nematode population both in the soil and root. This indicates that the damage caused to roots by *R. similis* restricted the infestation and multiplication of *M. incognita* to some extent.

The results of the three experiments have shown that both the nematode species affected the growth and productivity of the vines. The vines inoculated with *R. similis* alone or in combination with *M. incognita* exhibited symptoms such as foliar yellowing which intensified with time leading to defoliation and die back, typical of slow decline (slow wilt). The results corroborate the earlier reports that *R. similis* is the primary incitant of slow wilt/ pepper yellows (Ichinohe, 1976; Venkitesan, 1976; Ramana, Mohandas and Balakrishnan, 1987). *M. incognita* caused typical galls on the roots. *R. similis* feeds on cortical tissues and caused lesions. Many lesions coalesce and encircle the roots resulting in the disruption in the translocation of water and minerals. This resulted in disintegration and rotting of roots. Due to the repeated infestation by nematodes most of the fibrous roots were lost leaving only few thick main roots. The damage caused to the root system is

Table IX. *Root knot index (RKI), root lesion index (RLI) and nematode population in the black pepper vines inoculated with the nematodes*

Inoculum level	*Meloidogyne incognita*			*Radopholus similis*		
	R.K.I.	Nematode population		R.L.I.	Nematode population	
		Soil (100 cc)	Root (1 g)		Soil (100 cc)	Root (1 g)
Uninoculated control	0.00	Nil	Nil	1.00	Nil	Nil
MI 500	1.50	95.16	725.00	1.00	Nil	Nil
MI 1000	3.00	170.66	1633.33	1.00	Nil	Nil
RS 500	0.00	Nil	Nil	2.33	21.66	216.96
RS 1000	0.00	Nil	Nil	3.83	42.00	275.00
MI 500+RS 500 (S)	1.33	81.10	591.66	2.00	35.00	183.33
MI 500+RS 500 (20 DA)	1.50	66.16	533.33	2.16	18.16	208.33
RS 500+MI 500 (20 DA)	1.16	53.50	475.00	2.00	52.50	250.00
MI 1000+RS 1000 (S)	2.00	36.16	225.00	3.50	41.66	291.66
MI 1000+RS 1000 (20 DA)	2.33	46.83	358.33	3.66	53.50	283.33
RS 1000+MI 1000 (20 DA)	2.16	44.16	308.33	3.50	37.50	258.33

MI – *M. incognita;* RS – *R. similis;* S – Simultaneously; 20 DA – 20 days after first inoculation

reflected in the expression of above ground symptoms such as foliar yellowing, defoliation and die back.

These experiments were conducted in the fumigated field and soil mixture and care was taken to avoid contamination during the course of study. But is not unlikely that the experimental area could have been contaminated with fungal pathogens though not with nematodes during the course of four years under field conditions. Hence, it is suggested to investigate further the role of fungi such as *Phytophthora* sp. in increasing the severity of root damage in association with *R. similis* since there is no spatial separation of these two pathogens in the soil under natural conditions.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. M. K. Nair, former Director, Dr. A. Ramadasan, Officiating Director, National Research Centre for Spices and Dr. P.K. Koshy, Head, Nematology Division, CPCRI Regional Station, Kayangulam, for their valuable guidance and for providing necessary facilities for conducting the experiments.

REFERENCES

ANONYMOUS, 1986. Central Plantation Crops Research Institute, Annual Report for the year 1985 pp. 19–20.

BRIDGE, J. 1978. Plant nematodes associated with cloves and black pepper in Sumatra and Bangka; Indonesia, (O.D.M. Technical Report on visit to Indonesia 9–19 July, 1978) Ascot. Berks, U.K., Ministry of Overseas Development (1978) iii + 19 pp. (En) Imperial Coll. of London University, Silwood Park, Sounninghill, Ascot, Berks, U. K.

BUTLER, E. J. 1906. The wilt diseases of pigeon pea and pepper. *Agril. J.* India. **1**: 25–36.

CHRISTIE, J. R. 1957. The yellow disease of pepper and spreading decline of citrus. *Pl. Dis. Reptr.* **41**: 267–268.

D'SOUZA, G. I., KUMAR, A. C., VISWANADHAN, P. R. K. and SHAMANNA, H. V. 1970. Relative distribution and prevalence of plant parasitic nematodes in the coffee tracts of South-western India. *Indian Coffee.* **34**: 329–330 & 342.

FERRAZ, E. C. A. and SHARMA, R. D. 1979. Interaction and pathogenicity of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood 1949 and *Rotylenchulus reniformis* Linford and Oliveira 1940 on black pepper. *Revista Theobroma.* **9**: 45–53.

HUBERT, F. P. 1957. Diseases of some export crops in Indonesia. *Pl. Dis. Reptr.* **41**: 55–64.

ICHINOHE, M. 1976. Nematode problems of black pepper in Bangka Island, Indonesia. *Nematology Newsletter.* **22**: 2.

JACOB, J. A. and KURIAN, K. J. 1979. Nematodes associated with pepper in Kerala and the extent of damage done by *Meloidogyne incognita* on the crop. *Proc. PLACROSYM II,* 1979. pp. 31–38 (ed) C. S. Venkataram, Central Plantation Crops Research Institute, Kasaragod, India.

KOSHY, P. K., PREMCHANDRAN, D.. SOSAMMA, V. K. and PREMKUMAR, T. 1979. Effect of *Meloidogyne incognita* on black pepper. *Indian Phytopath.* **32**: 221–225.

KUMAR, A. C., VISWANADHAN, P. R. K. and D'SOUZA, G. I. 1971. A study of plant parasitic nematodes of certain commercial crops in coffee tracts of South India. *Indian Coffee.* **35**: 222–224.

LAMBERTI, F., ROHINI, H. M. and EKANAYAKE, K. 1983. Effect of some plant parasitic nematodes on the growth of black pepper in Sri Lanka. *Plant Protection Bull.* **31**: 163–166.

MOHANDAS, C. and RAMANA, K. V. 1983. Effect of different levels of *Meloidogyne incognita* on plant growth of two cultivars of black pepper *(Piper nigrum* L.) (Abstr.) All India Nematol. Symp. H. P. K. V. V., Solan, 24–26 May, 1983. pp. 9–10.

MUSTIKA, I. 1978. Observations on the relationship between nematode population and yellow disease on black pepper in Bangka. *Pemberitaan Lembaga Penelitian Tanaman Industri*, Bogor, Indonesia No. 30, pp. 11–22.

MUSTIKA, I. 1984. Effects of nematodes and fungi on the growth of pepper and yellow disease. *Pemberitaan Lembaga Penelitan Tanaman Industri*, Bogor, Indonesia 8: 28–37.

NAMBIAR, K. K. N. and SARMA, Y. R. 1977. Wilt diseases of black pepper. *J. Plant. Crops.* 5 : 92–103.

RAMANA, K. V. and MOHANDAS, C. 1987. Plant parasitic nematodes associated with black pepper *(Piper nigrum* L.) in Kerala. *Indian J. Nematol.* 17 : 62–66.

RAMANA, K. V., MOHANDAS, C. and BALAKRISHNAN, R. 1987. Role of plant parasitic nematodes in the slow wilt disease complex of black pepper *(Piper nigrum* L.) in Kerala. *Indian J. Nematol.* 17 : 225–230.

RAMANA, K. V., MOHANDAS, C. and RAVINDRAN, P. N. 1987. Reaction of black pepper germplasm to the burrowing nematode *(Radopholus similis). J. Plant. Crops* 15: 65–66.

TAYLOR, A. L. and SASSER, J. N. 1978. Biology, identification and control of root-knot nematodes. North Carolina State University, Raleigh, North Carolina, U. S. A. pp. 111.

TING, W. P. 1975. Plant pathology in peninsular Malaysia. *Rev. Pl. Path.* 54 : 297–305.

VECHT, J. VAN DER. 1950. Plant parasitic nematodes. pp. 16–45. In Karshoven, L. G. E. and Vecht, J. V. D. (ed) Diseases of cultivated plants in Indonesia, Vol. 1, (In Dutch), W. Van Hoeves-Gravenhage.

VENKITESAN, T. S. 1972. On the occurrence of plant parasitic nematodes associated with different crops in Cannanore district, Kerala, *Agric. Res. J. Kerala* 10 : 179–180.

VENKITESAN, T. S. 1976. Studies on burrowing nematode *Radopholus similis* (Cobb, 1893) Thorne, 1949 on pepper *(Piper nigrum* L.) and its role in slow wilt disease. Ph.D. thesis, University of Agricultural Sciences. Bangalore. pp. 118.

VENKITESAN, T. S. and SETTY, K. G. H. 1977. Pathogenicity of *Radopholus similis* to black pepper *(Piper nigrum* L.). *Indian J. Nematol.* 7 : 17–26.

WINOTO, S. R. 1972. Effect of *Meloidogyne* species on the growth of *Piper nigrum* L. *Mal. Agric. Res.* 1 : 86–90.

Journal of Plantation Crops 20 (Supplement) : 65-68 (1992)

SLOW DECLINE OF BLACK PEPPER (*PIPER NIGRUM* L.) AND ROLE OF PLANT PARASITIC NEMATODES AND *PHYTOPHTHORA CAPSICI* IN THE DISEASE COMPLEX

K.V. RAMANA, Y.R. SARMA and C. MOHANDAS[1]

National Research Centre for Spices, Calicut 673 012, Kerala

ABSTRACT

A field trial on management of slow decline of black pepper (*Piper nigrum* L.) in arecanut-black pepper mixed cropping system was taken up with soil application of phorate, neem cake and bavistin. Application of nematicide and neem cake was highly effective in reducing the populations of *Meloidogyne incognita* and *Radopholus similis*. However, treatments did not show clear evidence of remission of slow decline symptoms. Moreover, during the course of three years of trial the vine death ranged from 16.6 to 58.3 per cent in different treatments. *Phytophthora capsici* was isolated from feeder roots of disease affected vines. This suggests that *P. capsici* also may be playing a role in the slow decline complex. High percentage of mortality in bavistin treated vines is another indirect supporting evidence for *Phytophthora* involvement in root rot since this fungus is insensitive to bavistin. There appears to be a lack of spatial segregation of plant parasitic nematodes and *Phytophthora* under field conditions. The studies strongly indicate the need for integrated disease management to check both plant parasitic nematodes and *Phytophthora* and to boost the vigour and productivity of the pepper vines.

INTRODUCTION

Slow decline (slow wilt) and *Phytophthora* foot rot (quick wilt) of black pepper (*Piper nigrum* L.) are the major constraints in pepper production. These diseases are prevalent in all the pepper growing tracts in Kerala and Karnataka. Plant parasitic nematodes viz., *Meloidogyne incognita* and *Radopholus similis* were reported to be primarily responsible for slow decline (van der Vecht, 1950; Christie, 1957; Ichinohe, 1975 & 1976; Ting, 1975; Venkitesan and Setty, 1977; Mustika, 1978 and Ramana *et al.*, 1987). Pathogenicity tests conducted under simulated field conditions at National Research Centre for spices, Calicut, Kerala, have shown that both the nematode species caused significant damage to root system leading to foliar yellowing, defoliation and die back symptoms which are typical of slow decline syndrome (Mohandas & Ramana, Unpublished). In a pure plantation, application of phorate 10G @ 3 g a.i./vine, twice in a year significantly reduced the nematode population and improved the health of the vines (Anonymous, 1985). According to Hubert (1957) and Bridge (1978), *R. similis* was primarily responsible for the disease in Indonesia, but an association with fungus like *Fusarium* sp. was necessary to cause 'Yellows' disease. Nambiar and Sarma (1979) opined that the disease is complex in nature involving nematode fungal complex coupled with nutrient deficiency and soil moisture stress. Consistent association of *Fusarium* sp. with the roots of slow decline affected vines was reported (Nambiar and Sarma, 1977) though the role of the fungus in the disease was not understood then. In view of the known efficacy of bavistin in the control of *Fusarium* sp. and in order to get indirect information on the role of *Fusarium*, if any, field trial for disease management was undertaken to study the efficacy of phorate alone and in combination with bavistin in reducing the disease incidence in areca-pepper cropping system.

MATERIALS AND METHODS

The trial was conducted in an arecanut based black pepper plantation at Central Plantation Crops Research Institute, Regional Station, Vittal (Karnataka) during 1985-88. A total of 216 pepper vines in a contiguous block were selected for the experiment with the following treatments. The experiment was

[1]Present Address : Central Tuber Crops [illegible]

replicated thrice with a plot size of 12 vines. Recommended package of practices were adopted throughout the trial. Treatments : T_1 - Control (No treatment), T_2 - Phorate 10 G @ 1.5 g a.i./vine, T_3 - Phorate 10 G @ 3 g a.i./vine, T_4 - Bavistin 0.2% soil drench @ 5 l/ vine, T_5 - Phorate 10 G @ 3 g a.i./vine + Bavistin 0.2% soil drench @ 5 l/vine and T_6 - Neem cake @ 2 kg/vine.

The treatments were given twice a year, first application during May/June and second application during September/October. Root samples were collected every year during September/October prior to second application of treatments. Populations of *M. incognita* and *R. similis* in the roots were estimated. The roots were also tested for the presence of *Phytophthora* using a selective medium PVPH. Disease symptoms such as foliar yellowing was recorded every year on the basis of visual scoring on 0-3 (0=Healthy, 1=Mild foliar yellowing, 2=Medium foliar yellowing, and 3=Severe foliar yellowing).

RESULTS AND DISCUSSION

Population of plant parasitic nematodes viz., *M. icognita* and *R. similis* in the roots estimated during different years are given in Tables I and II respectively. Final population (pf) of root knot nematode was significantly reduced over initial population (pi) in the vines treated with phorate 10 G at both dosages alone or in combination with bavistin soil drenching and neem cake ranging from 47.8 to 69.8 per cent (Table I). Maximum reduction in the nematode population (69.8%) was observed in the vines treated with neem cake followed by phorate 10G 3 g a.i./vine. On the contrary, the population of root knot nematode had significantly increased in the vines treated with bavistin alone(68.4%) followed by untreated control (40.4%). Similar trend was observed with regard to the population of *R. similis* (Table II). Phorate at both the dosages significantly reduced the nematode populations. Nematode population has considerably increased in the vines treated with bavistin alone which was more than the population increase in the untreated control. However, neem cake showed less efficacy in suppressing the population of *R. similis*

Though there was significant reduction in the population levels of both the nematode species in the roots of pepper vines treated with phorate/neem

Table I. Populations of root knot nematode, *Meloidogyne incognita* in black pepper (per 5 g root)

Treatment	1985 (pi)	1986	1987	1988 (pf)	% age reduction (-) or increase (+) in the pf over pi
Control (untreated)	788.9	1251.4	1334.9	1107.4	+40.4
Phorate 10 G @ 1.5 g a.i/vine	815.8	927.6 (-25.9)	531.1 (60.2)	296.7 (-73.2)	- 63.6
Phorate 10 G @ 3.0 g a.i/vine	903.9	596.2 (-52.3)	440.6 (-67.0)	381.1 (-65.6)	- 57.8
Bavistin 0.2% @ 5l/vine	908.6	1298.0 (+3.7)	1451.8 (+8.8)	1530.0 (+38.2)	+ 68.4
Phorate 10 G @ 3 g a.i/+ Bavistin 0.2% @ 5l/vine	728.9	627.1 (-49.9)	449.4 (-66.3)	380.2 (-65.7)	-47.8
Neemcake @ 2 kg/vine	760.7	477.3 (-61.9)	284.7 (-78.7)	229.8 (-79.3)	-69.8
C.D. at 5%	NS	576	570	625	

pi = Initial population, pf = Final population

Figures in parentheses denote percent reduction (-) or increase (+) over untreated control

cake, there was no corresponding decrease in the foliar yellowing indices (Table III) and subsequent improvement in the health of the vines. Further the disease manifested severely during the course of three years of experimentation leading to death of vines ranging from 16.6 to 58.3 per cent in different treatments. The highest mortality of the vines was observed in the bavistin-treated vines alone with the highest percentage of positive isolation of *P. capsici* from the roots. This might be due to the fact that bavistin

Table II. Population of burrowing nematode, *Radopholus similis* in black pepper (per 5g of root)

Treatment	1985 (pi)	1986	1987	1988 (pf)	% reduction (-) or increase (+) in the pf over pi
Control (untreated)	462.2	797.3	889.6	682.7	+ 71.0
Phorate 10 G @ 1.5 g a.i/vine	351.2	270.8 (-66.0)	143.7 (-88.8)	162.8 (-76.2)	- 45.0
Phorate 10 G @ 3 g a.i/vine	413.3	236.1 (-70.4)	49.6 (-94.4)	94.5 (-86.2)	- 69.7
Bavistin 0.2% @ 5l/vine	312.8	535.3 (-32.9)	603.9 (-32.1)	1107.6 (+62.2)	+ 139.3
Phorate 10 G @ 3 g a.i. + Bavistin 0.2% @ 5l/vine	248.9	246.8 (-69.1)	179.5 (-79.8)	181.5 (-73.4)	- 18.5
Neemcake @ 2 kg/vine	343.7	388.7 (-51.3)	204.4 (-77.0)	396.5 (-41.9)	- 4.1
C.D. at 5%	N.S.	280	349	377	..

pi = Initial population, pf = Final population
Figures in parentheses denote percent reduction (-) or increase (+) over untreated control

Table III. Foliar yellowing and mortality of black pepper in the experimental plot

Treatments	Foliar yellowing index				Mortality of the vines		% isolation of *P. capsici* (out of 50 root bits tested)
	1985	1986	1987	1988	No.	%	
Control (Untreated)	1.64	1.42	1.89	1.92	10	27.7	48
Phorate 10G @ 1.5 g a.i./vine	1.67	1.39	1.28	1.66	6	16.6	40
Phorate 10G @ 3 g a.i/vine	1.64	1.11	1.19	1.69	16	44.4	76
Bavistin 0.2% @ 5l/vine	1.61	1.18	1.81	2.24	21	58.3	60
Phorate 10G @ 3g a.i. + Bavistin 0.2% @ 5l/vine	1.61	1.31	1.16	1.51	10	27.7	36
Neemcake @ 2 kg/vine	1.53	1.22	1.45	1.42	7	19.4	48

Foliar yellow on 0-3 scale (0=Healthy; 1=mild; 2=medium & 3=severe)

is known to suppress fungi like *Fusarium* sp. only and not *Phytophthora*. This also indirectly indicates that *Fusarium* sp. has no role in root rot and death of vines. The increased vine death in bavistin-treated vines might be due to its ineffectiveness to control *Phytophthora*. Further studies are needed to understand the microbiological changes in the soil in relation to *Phytophthora* population to see whether there is any disturbance in the native population of natural antagonists of *Phytophthora* present in bavistin-treated plots. All the vines irrespective of treatment gave positive isolation of *Phytophthora* indicating its role in causing root damage and subsequent expression of symptoms. Anandaraj *et al.* (1988) indicated that when the infection of *P. capsici* is restricted to certain portion of the root system, initially the vines express symptoms such as foliar yellowing and defoliation. These studies indicated that infection by plant parasitic nematodes and fungi could result in root degeneration leading to slow decline. Since spatial segregation of plant parasitic nematodes and *Phytophthora* cannot exist under field condition, the study strongly suggests for an integrated disease management to check plant parasitic nematodes and *P. capsici* and to boost up the health and vigour of black pepper vine.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. M.K. Nair, Director, Central Plantation Crops Research Institute, Kasaragod and Joint Director, CPCRI Regional Station, Vittal for providing facilities and to Dr. K.B. Abdul Khader, Sr. Scientist (Agronomy), CPCRI Regional Station, Vittal, for all the help given throughout. Thanks are due to Dr. A. Ramadasan, Director, National Research Centre for Spices, Calicut for the encouragements.

REFERENCES

ANANDARAJ, M., RAMACHANDRAN, N. and SARMA, Y.R. 1988. Epidemiology of foot rot disease of black pepper (*Piper nigrum* L.) in India. Paper presented at the International Workshop on Wilt Diseases of Black Pepper. 27-29 October, 1988. Panaji, Goa.

ANONYMOUS. 1985. Central Plantation Crops Research Institute. 1985. Annual Report for 1984. Kasaragod, India. p. 16.

BRIDGE, J. 1978. Plant nematodes associated with cloves and black pepper in Sumatra and Bangka, Indonesia. (ODM Technical Report on visit to Indonesia, 9-19 July, 1978). Ascot, Berks, U.K., Ministry of Overseas Development (1978). iii + 19 pp. (Fn) Imperial College of London University; Silwood Park, Sunninghill, Ascot, Berks, U.K.

CHRISTIE, J.R. 1957. The yellow disease of pepper and spreading decline of citrus. *Plant Dis. Reptr.* 41: 267-268.

HUBERT, F.P. 1957. Diseases of some export crops in Indonesia. *Plant Dis. Reptr.* 41 : 55-64.

ICHINOHE, M. 1975. Infestation of black pepper vines by the root knot nematode, *Meloidogyne incognita* at Tom-Acu, Pará, Brazil. *Jap. J. Nematol.* 5 : 36-40.

ICHINOHE, M. 1976. Nematode problems of black peppers in Bangka Island, Indonesia. *Nematology Newsletter* 22 : 2.

MUSTIKA, I. 1976. An observation on the relationship between nematode population and yellow disease on black pepper in Bangka. Pemberitaan Lembaga Penelitian Tanaman Industri, Bogor, Indonesia No. 30 11-22.

NAMBIAR, K.K.N. and SARMA, Y.R. 1977. Wilt diseases of black pepper. *J. Plants Crops* 5 : 92-103.

NAMBIAR, K.K.N. and SARMA, Y.R. 1979. Factors associated with slow wilt of pepper. Proc. PLACROSYM-2. 1979. pp. 348-358.

RAMANA, K.V., MOHANDAS, C. and BALAKRISHNAN, R. 1987. Role of plant parasitic nematodes in the slow wilt disease complex of black pepper (*Piper nigrum* L.) in Kerala. *Indian J. Nematol.* 17 : 225-230.

TING, W.P. 1975. Plant Pathology in Peninsular Malaysia. *Rev. Pl. Path.* 54 : 297-305.

VECHT, J. Van Der. 1950. Op Plantenparasiterende achtjes (Tylenchidae). In *De plagen van de cultuurgewassen in Indonesie*, p. 16-42. 's-Gravenhage, W. van Hoeve.

VENKITESAN, T.S. and NAIR, K.G.H. 1977. Pathogenicity of *Radopholus similis* to black pepper (*Piper nigrum*). *Indian J. Nematol.* 7 : 17-26.

Efficacy of *Paecilomyces lilacinus* (Thom.) Samson in suppressing nematode infestations in black pepper (*Piper nigrum* L.)[1]

K V RAMANA
National Research Centre for Spices
Calicut - 673 012, Kerala, India.

ABSTRACT

The efficacy of *Paecilomyces lilacinus* in suppressing root-knot nematode (*Meloidogyne incognita*) and burrowing nematode (*Radopholus similis*) infestations in black pepper (*Piper nigrum*) was studied. Though the fungus could not effect absolute control of nematodes, it signficantly suppressed nematode infestation and increased total root mass production. The fungus was more effective in suppressing *M. incognita* than *R. similis*.

Key words : black pepper, *Meloidogyne incognita*, nematodes, *Paecilomyces lilacinus, Piper nigrum, Radopholus similis*.

Introduction

The production and productivity of black pepper (*Piper nigrum* L.) in India is severely affected due to infestation by plant parasitic nematodes viz., *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949 and *Radopholus similis* (Cobb 1893) Thorne 1949 leading to 'slow decline'. These two nematode species are widely distributed in all major black pepper growing areas (Kumar, Viswanathan & D'Souza 1971; Venkitesan 1972; Jacob & Kuriyan 1979; Sundararaju, Koshy & Sosamma 1979; Ramana & Mohandas 1987& 1989). Pathogenicity tests conducted under simulated field conditions showed that *M. incognita* and *R. similis* caused significant reduction in growth and productivity of black pepper vines (Mohandas & Ramana 1991).

Though several nematicides are effective in checking nematode infestations in black pepper (Nambiar & Sarma 1977; Venkitesan & Charles 1979; Venkitesan & Setty 1979; Anonymous 1985; Mohandas & Ramana 1987), their usage is limited because of high cost, difficulty in handling, environmental pollution and health hazards due to continuous application. Hence there is need for utilising biocontrol agents which form a major component in integrated nematode managment programmes.

In an earlier effort on biocontrol of nematodes infesting black pepper, four

[1]Contribution No. 190 of National Research Centre for Spices, Calicut - 673 012, Kerala, India.

species of vesicular arbuscular mycorrhizae were effective in suppressing nematode infestations which was on par with phorate treatment (Anandaraj, Ramana & Sarma 1991). *Paecilomyces lilacinus* (Thom.) Samson, a soil inhabiting hypomycetes fungus was reported highly effective in suppressing root-knot nematodes (Jatala 1985 & 1986; Ibrahim *et al.* 1987; Shahzad & Ghaffar 1987; Cabanillas, Barker & Daykin 1988; Khan & Hussain 1988; Sharma *et al.* 1988), *Tylenchulus semipenetrans* (Reddy 1988), *Rotylenchulus reniformis* (Khan & Hussain 1988; Reddy & Khan 1988 & 1989) and cyst nematodes (Franco & Bocangel 1981; Saifullah & Saeed 1988). Hence a study was conducted to assess the efficacy of *P. lilacinus* in suppressing infestations of *M. incognita* and *R. similis* in black pepper.

Materials and methods

Single node rooted cuttings of black pepper hybrid Panniyur-1 were planted singly in 20 cm dia earthern pots filled with 6.5 kg fumigated soil. Three months after planting, the following treatments were imposed in five replications: 1) Control 2) Phorate 10G 3) *M. incognita* 4) *R. similis* 5) *P. lilacinus* 6) *P. lilacinus* + *M. incognita* 7) *P. lilacinus* + *R. similis* 8) *M. incognita* + *R. similis* 9) *P. lilacinus* + *M. incognita* + *R. similis* 10) *M. incognita* + *R. similis* + Phorate 10G. *P. lilacinus* was mass cultured on rice meal medium in conical flasks. Ten day old culture was blended and made up to 200 ml suspension with sterlie distilled water. This suspension was incorporated into the root zone @ 20 ml/pot. Freshly hatched second stage juveniles of *M. incognita* (2000/pot) from egg masses collected from black pepper roots and *R. similis* (150/pot) were introduced into the root zone 20 days after fungal inoculation. Phorate 10G @ 3g/pot was applied at quarterly intervals. All the plants were arranged in a Randomized Block Design in a net house. Twelve months after planting, the plants were depotted, washed thoroughly and fresh weights of shoot and root were recorded. Root-knot index (RKI) and root lesion index (RLI) of individual plants were recorded.

Results and discussions

Both the nematode species caused significant reductions in biomass production (Table 1). Reduction in fresh weights of shoot and root were 39.2 and 18.1 per cent and 64.8 and 31.6 per cent due to *R. similis* and *M. incognita* inoculations, respectively. Combined inoculation of both the nematodes caused 61.2 and 74.5 per cent reduction in fresh weights of shoot and root, respectively. Fungus inoculation resulted in significant increase in biomass production of shoot and root even though the plants were inoculated with nematodes. Loss in root mass was reduced from 64.8 to 47.3 per cent and 31.6 to 21.2 per cent due to *R. similis* and *M. incognita*, respectively, when these plants were inoculated with the fungus.

RLI was maximum (3.6) in plants inoculated with *R. similis* alone followed by combined inoculation of both nematode species (3.4). When the fungus was incorporated, RLI was significantly reduced. Similarly, RKI was brought down from 4.4 in plants inoculated with *M. incognita* alone to 2.4 in plants treated with the fungus. However, phorate application was also highly effective in supressing nematodes.

The study indicates that though the

fungus could not suppress completely, it significantly reduced nematode infestation and thereby increased root mass. The fungus showed greater efficacy on *M. incognita* than *R. similis*. The specificity of *P. lilacinus* as an egg parasite of root-knot nematodes might be the reason for reduction in RKI in plants inoculated with *M. incognita* and *P. lilacinus*. The study also further confirmed the efficacy of *P. lilacinus* in suppressing nematodes infesting black pepper. However, the recent report that *P. lilacinus* is a human pathogen (Kerry 1987) indicates the necessity to use the fungus cautiously for nematode management.

Table 1. Effect of *Paecilomyces lilacinus* in suppressing nematode infestations in black pepper

Treatment	Fresh weight (g)		RKI	RLI
	Shoot	Root		
Control	267.54	68.22	1.4	1.4
Phorate	296.74 (+10.9)	71.84 (+5.3)	1.0	1.0
Meloidogyne incognita	219.06 (-18.1)	46.64 (-31.6)	4.4	1.0
Radopholus similis	162.66 (-39.2)	23.98 (-64.8)	1.0	3.6
Paecilomyces lilacinus	275.28 (+2.9)	73.84 (+8.2)	1.0	1.0
M. incognita + *P. lilacinus*	230.72 (-13.8)	82.72 (+21.2)	2.4	1.0
R. similis + *P. lilacinus*	(190.04) (-28.9)	35.96 (-47.3)	1.0	2.4
M. incognita + *R. similis*	[illegible] (-61.2)	17.40 (-74.5)	2.8	3.4
M. incognita + *R. similis* + *P. lilacinus*	249.64 (-6.7)	60.18 (-11.8)	2.0	2.2
M. incognita + *R. similis* + Phorate	268.78 (+0.5)	64.56 (-5.4)	1.6	1.8
LSD (P=0.05)	38.03	16.63	0.6	0.6

RKI : 1 = No galls; 2 = Mild galling; 3 = Medium galling; 4 = High galling; 5 = Very high galling and root rotting

RLI : 1 = No lesion; 2 = Few isolated lesions; 3 = Many lesions, few coalescing, root tips damaged; 4 = Many lesions, coalescing, encircling the main root, lateral roots damaged; 5 = Whole root system damaged

Figures in parentheses denote percent reduction (-) / increase (+) over control

Pathogenicity of *Meloidogyne incognita* and *Radopholus similis* on black pepper (*Piper nigrum* L.). J. Plant. Crops 19 : 41-53.

Nambiar K K N & Sarma Y R 1977 Wilt diseases of black pepper. J. Plant. Crops 5 : 92-103.

Ramana K V & Mohandas C 1987 Plant parasitic nematodes associated with black pepper (*Piper nigrum* L.) in Kerala. Indian J. Nematol. 17 : 62-66.

Ramana K V & Mohandas C 1989 Endoparasitic nematodes infecting roots of black pepper (*Piper nigrum* L.) in two districts of Karnataka, India. Int. Nematol. Network Newsl. 6 : 33-35.

Reddy P P 1988 Nematode Management in Horticultural Crops. Tech. Bull. 4. Indian Institute of Horticultural Research, Bangalore.

Reddy P P & Khan R M 1988 Evaluation of *Paecilomyces lilacinus* for the biological control of *Rotylenchulus reniformis* infecting tomato compared with carbofuran. Nematol. medit. 16 : 113-116.

Reddy P P & Khan R M 1989 Evaluation of biocontrol agent *Paecilomyces lilacinus* and carbofuran for the management of *Rotylenchulus reniformis* infecting brinjal. Pakistan J. Nematol. 7 : 55-59.

Saifullah Gul A & Saeed M 1988 Efficacy of *Paecilomyces lilacinus* against golden nematode (*Globodera rostochiensis*) in Pakistan. Int. Nematol. Network Newsl. 5 : 20-22.

Shahzad S & Ghaffar A 1987 Field application of *Paecilomyces lilacinus* and Furadan for the control of root knot disease of okra and mung. Int. Nematol. Network Newsl. 4 : 33-34.

Sharma, J L, Trivedi P C, Sharma M K & Tiagi B 1988 Fungus *Paceilomyces lilacinus* as a biocontrol agent of root-knot nematode *Meloidogyne incognita*. Indian Zoologist 12 : 259-262.

Sundararaju P, Koshy P K & Sosamma V K 1979 Survey of plant parasitic nematodes associated with spices in Kerala and Karnataka. In: Venkataram C S (Ed.) Proc. PLACROSYM II (pp. 39-44). Central Plantation Crops Research Institute, Kasaragod, Kerala.

Venkitesan T S 1972 On the occurrence of plant parasitic nematodes associated with different crops in Cannanore district, Kerala. Agric. Res. J. Kerala 10 : 179-180.

Venkitesan T S & Charles J S 1979 A note on the chemical control of nematodes infesting pepper vines in Kerala. In : Venkataram C S (Ed.) Proc. PLACROSYM II (pp.27-30). Central Plantation Crops Research Institute, Kasaragod, Kerala.

Venkitesan T S & Setty K G H 1979 Control of the burrowing nematode *Radopholus similis* on black pepper. Pesticides 13 : 40-42.

Journal of Plantation Crops 24 (Supplement) : 166-170 (1996)

ROLE OF *PHYTOPHTHORA CAPSICI* IN THE ETIOLOGY OF SLOW DECLINE DISEASE OF BLACK PEPPER (*PIPER NIGRUM* L.)*

M. ANANDARAJ, K.V. RAMANA and Y.R. SARMA

National Research Centre for Spices, Calicut - 673 012, Kerala, India

ABSTRACT

Feeder root loss in black pepper (*Piper nigrum* L.) caused by *Phytophthora capsici, Radopholus similis and Meloidogyne incognita* either alone or in combination leads to slow decline. When the feeder root infection by *P. capsici* reaches the collar through roots, it results in foot rot disease.

INTRODUCTION

Among the diseases of black pepper (*Piper nigrum* L.) Phytophthora foot rot and slow decline are very serious. Foot rot is caused by *Phytophthora capsici,* whereas slow decline is caused by plant parasitic alone or in association with nematodes and fungi (Nambiar and Sarma 1977; Sarma et. al. 1991; 1992 & Ramana *et. al.* 1987; 1992). Two species of plant parasitic nematodes *viz., Radopholus similis* and *Meloidogyne incognita* were identified as primary incitants of slow decline (Ramana, 1991; Ramana & Mohandas 1987; Mohandas & Ramana, 1988). However, feeder root loss caused by *P. capsici* was also reported to lead to slow decline symptoms (Anandaraj *et. al.* 1991; 1994; Ramana *et. al.* 1992). In order to understand the combined effect of root damage caused by *Phytophthora* and nematodes the present investigation was carried out under simulated field conditions in microplots.

*Contribution No.211 of National Research Centre for Spices, Calicut - 673 012.

MATERIALS AND METHODS

Black pepper var. Panniyur-1 vines were raised in microplots. Each microplot consisted of 1 M^3 cement tub filled with nursery mixture (forest soil : sand : FYM, 3 : 1 : 1) fumigated with methyl bromide. These tubs were buried in soil upto the brim with drain holes pointing towards channel dug on either side of the tubs. Black pepper vines were allowed to trail on teak poles planted next to each tub. When the plants were one year old, they were inoculated with pathogens as per the following treatments.

T_1 *Phytophthora capsici* (P.c)

T_2 *Radopholus similis* (R.s)

T_3 *Meloidogyne incognita* (M.i)

T_4 P.c + R.s

T_5 P.c + M.i

T_6 M.i + R.s

T_7 P.c + R.s + M.i

T_8 Control

T_9 Control with Phorate 30g and Ridomil Mancozeb 6.25g/plant

The design followed was completely randomized design with six individual vines as replications.

P. capsici isolated from black pepper was inoculated on detached black pepper leaves and the infected leaves were cut into bits and mixed with autoclaved soil in the ratio of 1:9, to get a soil infested with *P. capsici.* This infested soil was used @ 250 cc/vine.

M. incognita infested black pepper roots were taken from the field, egg masses separated and were allowed to hatch. For each vine 1000 second stage larvae were used as inoculum. *R. similis* collected from infested black pepper roots was cultured on carrot discs and 250 nematodes/vine were used as inoculum. These 3 pathogens were inoculated separately and in combination as per the treatment simultaneously.

Observations on the aerial symptoms viz., foliar yellowing, defoliation and vine death were recorded at monthly intervals for two years. At the end of second year after inoculation, the surviving vines were uprooted, indexed for foliar yellowing, root rot, root lesions and root galls. The visual scoring for various indices are as follows.

a) Foliar yellowing

0 = No yellowing

1 = Upto 25% leaves yellow

2 = 26-50% leaves yellow

3 = 51-75% leaves yellow

4 = >75% leaves yellow

b) Root knot index (*M. incognita*)

0 = No galls

1 = few galls

2 = galls on secondary & tertiary roots

3 = galls on primary, secondary & tertiary roots

c) Root lesion index (*R. similis*)

1 = No lesion

2 = Few isolated lesions

3 = Many lesions

4 = Lesions coalescing, lateral roots damaged

5 = Whole root system affected

d) Root rot index (*P. capsici*)

0 = No roting

1 = Upto 25%, feeder roots rotten

2 = Upto 50% "

3 = Upto 75% "

4 = > 75% "

RESULTS AND DISCUSSION

The vines inoculated with *P. capsici* and *R. similis* either alone or in combination started exhibiting declining symptoms two months after inoculation of the fungus/nematodes. Foliar yellowing was followed by defoliation. In some cases with the onset of monsoon, fresh foliage emerged and the intensity of yellowing was reduced. About 50% of the defoliated vines succumbed to infection which were inoculated with *P. capsici* or *R. similis* either alone or in combination. Foliar yellowing was similar both with *P. capsici* and *R. similis* whereas *M. incognita* inoculated plants showed interveinal chlorosis. Mortality of the vine was upto 66.6% in the treatment involving *P. capsici* and *R. similis.* The treatment involving all the three pathogens, had 50% mortality of vines and the remaining plants showed severe root rot and defoliation (Table I). When the experiment was concluded 2 years after inoculation, none of the

Table I. Effect of *P. capsici* and *M. incognita* on black pepper

Treatment	Mortality (%)	Yellowing index (0-4)	Defoliation index (0-4)	Shoot weight (g)		Root weight (g)		Root lesion index (1-5)	Root knot index (0-3)	Root rot Index 0-4	
				Fresh	Dry	Fresh	Dry			Feeder root	Main root
P.c	50.0	1.5	1.5	1710	617.0	237.5	66.5	0.0	0.0	3.0	3.0
R.s	50.0	0.6	1.6	1360	486.9	261.0	73.0	3.0	0.0	0.0	0.0
M.i	16.6	1.2	1.2	2761	988.4	445.0	124.6	0.0	2.2	2.0	0.0
P.c + R.s	66.6	1.5	1.5	1915	685.6	332.5	93.1	2.5	0.0	2.0	2.5
P.c + M.i	16.6	0.8	1.8	2765	989.8	330.8	92.6	0.0	2.2	2.4	1.6
M.i + R.s	16.6	1.0	1.5	2537	908.2	145.0	40.6	3.0	2.7	2.0	1.8
P.c + R.s + M.i	50.0	2.0	3.0	735	263.1	74.3	20.8	3.3	1.3	3.0	3.0
Control	0.0	0.0	0.0	3600	1288.8	465.0	130.2	0.5	0.5	.0	0.8
Control (Ph + RMZ)	0.0	0.0	0.0	3898	1395.5	802.2	224.7	0.0	0.0	0.2	0.2

surviving vines showed healthy root system. The fresh and dry weight of the vines also indicated that minimum weight was in the treatment involving all the pathogens (Table I) due to root damage and consequent reduction in dry matter production.

The initial symptom expressed in the vines inoculated with *P. capsici* or *R. similis* was foliar yellowing similar to nutritional deficiency. Since both the pathogens infected and caused damage to feeder roots leading to reduction in the absorptive area, the vines might have experienced shortage of nutrients and hence the deficiency symptoms. During monsoon which triggers the production of new feeder roots and foliage, the yellowing was less and some of the affected vines apparently revived. Since the symptoms were similar to nutrient deficiency it was thought to be caused by nutritional imbalances (Nambiar & Sarma, 1977.) At the end of monsoon when soil moisture depleted, with damaged root systems, the vines were unable to cope up with transpirational demand, leading to defoliation. When *P. capsici* infects and enters through the feeder root, it also infects older roots which culminate in collar infection. (Anandaraj *et. al.*, 1994). Whereas in *R. similis* after the tender feeder roots are damaged it also affects the main roots. *M. incognita* prefers the tender zone of root elongation and results in formation of galls. When the feeder roots are damaged the uptake of nutrients is also affected resulting in stunted growth. The present study has established that the nature of damage is caused by these three pathogens alone and in combination. This varying degrees of root damage led to declining symptoms. The degenerated root system under moisture stress which is unable to cope up with absorption might have resulted in starvation symptoms. In the present study under field simulated microplots, eventhough enough moisture and nutrients were available, the plants exhibited declining symptoms due to feeder root damaged by these three pathogens. This confirms the primary role of these root pathogens in causing slow decline and precludes the role of nutrient deficiency and moisture stress.

REFERENCES

ANANDARAJ, M., RAMACHANDRAN, N. and SARMA, Y.R. 1991. Epidemiology of foot rot disease of black pepper (*Piper nigrum* L.) in India. *In Black Pepper Diseases* - Proc. International Pepper Community Workshop on Black pepper Diseases 27-29 October 1988, Goa, India. (Eds) Y.R. Sarma & T. Premkumar, pp.110-135. National Research Centre for Spices, Calicut.

ANANDARAJ, M., SARMA, Y.R. and RAMACHANDRAN, N. 1994 *Phytophthora* root rot of black pepper in relation to age of the host and its culmination in foot rot. *Indian Phytopath* **47**: 203-206

MOHANDAS, C. and RAMANA, K.V. 1988. Management of slow wilt disease of black pepper. *Cardamom* **21**: 19-21

NAMBIAR, K.K.N. and SARMA, Y.R. 1977. Wilt disease of black pepper. *J. Plant. Crops.***5**: 92-103

NAMBIAR, K.K.N and SARMA, Y.R. 1979 Factors associated with slow wilt of pepper. In: *Proc. PLACROSYM-II* (Ed) C.S. VENKATARAM, pp. 386-397. Indian Society for Plantation Crops, Central Plantation Crops Research Institute, Kasaragod, India

RAMANA, K.V. 1991 Slow decline disease of black pepper (*Piper nigrum* L.) in India. In:, *Black pepper diseases,* Proc. International Pepper Community

Workshop on black pepper diseases 27-29 October 1988, Goa, India (Eds) Y.R. Sarma and T. Premkumar pp. 136-157. National Research Centre for Spices, Calicut.

RAMANA, K.V. and MOHANDAS, C. 1987. Plant parasitic nematodes associated with black pepper (*Piper nigrum* L.) in Kerala. *Indian J. Nematol.* **17**:62-66.

RAMANA, K.V., MOHANDAS, C. and BALAKRISHNAN, R. 1987. Role of plant parasitic nematodes in the slow wilt disease complex of black pepper (*Piper nigrum* L.) in Kerala, *Indian J. Nematol.* **17**: 225-230

RAMANA, K.V. SARMA, Y.R. and MOHANDAS, C. 1992. Slow decline of black pepper (*Piper nigrum* L.) and role of plant parasitic nematodes and *Phytophthora capsici* in the disease complex. *J. Plant Crops* **20** (suppl.) : 65-68.

SARMA, Y.R., RAMACHANDRAN, N. and ANANDARAJ, M. 1991. Black pepper diseases in India. In: Black pepper diseases. *Proc. International Workshop on black pepper diseases* 26-27 October, 1988, Goa, India (Eds) Y.R. Sarma & T. Premkumar, pp.55-101. National Research Centre for Spices, Calicut.

SARMA, Y.R., ANANDARAJ, M. and RAMACHANDRAN, N. 1992. Recent advances on *Phytophthora* foot rot research in India and the need for holistic approach pp 133-143. In : *proceedings of International Workshop on Black Pepper Diseases.* Bandor, Lampung Indonesia - 3-4 December 1991 (Eds. P. Wahid D. Stepu, S. Deciyanto and U. Suparman) Agency for Agricultural Research and Development, Research Institute for Spices and Medicinal Crops Bogor - Indonesia.

REFERENCES

Cobb, N.A. (1918). *Agric. Tech. Circ. Bur. Pl. Ind. U.S. Dep. Agric.* No. 1, 48 pp.

Hooper, D.J. (1970). *Tech. Bull. No. 2 Min. Agric. Fisheries* and Food. London: 39 pp.

Mani, A. & Har, D.S. (1989). *Indian J. Nematol.* **19**: 272-73.

Mani, A, Harinath Naidu, P& Madhavchari, S. (1987). *Int. Nematol. Network News letter* **4**: 13-18.

Patel, D.J., Makaida, B.M. & Shah, H.M. (1982). *Indian J. Nematol.* **12**: 168-71.

Patel, H.V., Patel, D.J. & Patel, B.A. (1989). *Int. Nematol.* Network Newsl. **6** : 24.

Routaray, B.N. & Das, S.N. (1982). *Indian J. Nematol.* **12**: 179-80.

Routaray, B.N., Sahoo, H. & Das, S.N. (1987). *Indian J. Nematol.* **17** : 122.

A note on the incidence of *Rotylenchulus reniformis* in cardamom plants

SANTHOSH J.EAPEN

National Research Centre for Spices,
Cardamon Research Centre, Appangla, Mercara, Karnataka- 571 201

Small cardamom, *Elettaria caramomum* is an important spice crop of India. About 20 genera of plant parasitic nematodes are reported to be associated with cardamom (Eapen, 1991). Among them, the root knot nematodes are the most important and widely distributed nematode pests. Recently high population (58-68/100g soil) was observed in a cardamom nursery of reniform nematode. D'Souza *et al.* (1970) though reported the presence of *Rotylenchulus reniformis* in soil around cardamom but no reports are available on their presence in cardamom roots. Cardamom seedlings raised in the above nursery site were uprooted at 4-5 leaf stage, washed thoroughly and the roots were stained with acid fuchsin (Byrd *et al.*, 1983). Mature females were seen protruding from these roots with their heads embedded inside root cortex. Ten such females were teased out from these roots and the measurements were taken to confirm their identity. The females were identified as *Rotylenchulus reniformis*. Occasionally eggs were also seen inside the gelatinous matrix produced by the females. The number of mature females varied from 7-16 per gram root. Moderately thick and brown cortical thickening were observed on roots at the point of nematode entries.

REFERENCES

Byrd, D. W. Jr., Kirkpatrick, T. & Barker, K.R. (1983). *J. Nematol.* **15**: 142-143.

D'Souza, G.I., Kumar, A.C., Kasiviswanathan, P.R. & Shamanna, H.V. (1970). *Indian Coffee* **34**: 1-3.

Eapen, S.J. (1991). *Spice India* **4**: 6-12.

EFFECT OF *MELOIDOGYNE INCOGNITA, PYTHIUM APHANIDERMATUM* AND *RALSTONIA SOLANACEARUM* ALONE AND IN COMBINATIONS IN GINGER

K.V. Ramana, S.J. Eapen and Y.R. Sarma

Indian Institute of Spices Research
Calicut-673 012, Kerala

ABSTRACT : India is a major producer and exporter of ginger (*Zingiber officinale* Rosc.). The crop suffers serious losses due to several pests and diseases. Root-knot nematodes (*Meloidogyne* spp.) are important nematode pests of ginger. Rhizome rot/ soft rot caused by *Pythium aphanidermatum* and bacterial wilt caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum*) are serious constraints in ginger production. Preliminary surveys indicated that the severity of these diseases was more in the presence of root -knot nematodes. A series of pot culture experiments were conducted to find out the individual and interactive effects of *M. incognita* in ginger.

M. incognita caused 29.60 to 33.35% yield loss at Pi = 0.2 to 2.0/100 cc. soil Though the nematode showed no role in the bacterial wilt, it had synergistic effect in rhizome rot/soft rot of ginger, particularly when nematodes were inoculated to the plants prior to fungi inoculation. The damage to the crop was more and the onset of the disease was early when *M. incognita* was inoculated 40 days prior to fungal inoculation.

Keywords : Ginger, Interactions, *Meloidogyne incognita, Pythium aphanidermatum, Ralstonia solanacearum, Zingiber officinale.*

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is one of the export-oriented crops. India is a major producer and exporter of ginger. Plant parasitic nematodes and diseases like rhizome rot/soft rot and bacterial wilt are major constraints for ginger cultivation. Among several nematodes reported on ginger, root knot nematodes (*Melodigoyne incognita*) is most predominant in all ginger growing areas (Colbran, 1961; Charles and Kuriyan, 1980; Huang, 1966; Haynes et al., 1973; Kulkarni and Jain, 1965; Mammen, 1973; Milne, 1979; Routaray et al., 1987a, 1987b; Sundararaju et al., 1980). The nematodes causes significant reduction in growth and yield of ginger ranging from 46.40% (Charles and Kuriyan, 1980) to 74.10% (Sudha and Sundararaju et al., 1986). Besides causing damage on their own way, plant parasitic nematodes have positive role in the incidence and severity of many diseases caused by fungi and bacteria. In ginger, the incidence of rhizome rot/soft rot caused by *Pythium aphanidermatum* was found to be more severe when rhizomes were infested with *M. incognita* in Himachal Pradesh (Dohroo et al., 1987). However, others did not observe such interactions between fungi and nematodes in ginger (Doshi and Mathur, 1987; Lanjewar and Shukla, 1985). Samuel and Mathew (1986) reported that bacterial wilt caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum*) was influenced by the presence of *M. incognita*. Pot culture experiments were conducted to assess the pathogenic effect of *M. incognita* in ginger and its interactions in the incidence and severity of rhizome rot/soft rot caused by *Pythium aphanidermatum* and bacterial wilt caused by *R. solanacearum*.

MATERIALS AND METHODS

A. Pathogens

1. *Meloidogyne incognita* (Kofoid & White) Chitwood; Pure culture of the root knot nematode, M. incognita was maintained on ginger. Egg masses were collected, surface sterilized with sodium hypochlorite solution (0.1%) and allowed for hatching into distilled water. Freshly hatched second stage juveniles were used for inoculations.
2. *Pythium aphanidermatum* (Edson) Fitzp: The fungus was cultured on liquid medium (Potato Dextrose Broth) in 100 ml. conical flasks. The mycelial mat from 5-day-old cultures from each flask was blended in 200 ml of distilled water and was used for inoculation.
3. *Ralstonia solanacearum* (Smith) Smith : The bacterium was cultured on nutrient agar in petriplates. After 48 hrs the bacterial growth from each petriplate was scrapped into 200 ml distilled water and mixed thoroughly. Ten ml of this bacterial suspensions was inoculated to each pot as per treatments.

B. Effect of *M. incognita* on the growth and yield of ginger

Seed rhizomes of ginger variety, "Himachal" were sown in earthern pots (8" dia.) filled with sterilized soil mixture (5l/pot) @ 30g/pot. One month after germination, 40 pots having plants with uniform growth were selected for the experiment. The plants were inoculated with freshly hatched second stage juveniles @ 0, 0.2, 2.0, 20.0 and 200.0 nematodes/100 cc. soil in 8 replications. The pots were arranged in R.B.D. in a net house and watered regularly. Nine months after sowing, the plants were harvested and observations on growth characteristics of ginger and nematode multiplication in roots were recorded.

C. Effect of *M. incognita, P. aphanidermatum* and *R. solanacearum* alone and in combinations on the incidence of diseases

Seed rhizomes of ginger variety, "Himachal" were sown @ 30g in plastic pots (6" dia.) containing sterilized soil mixture (1 l/pot). One month after sowing, 40 plants with uniform growth were selected and the following treatments were imposed in 4 replications.

T1 - No pathogens (Control)

T2 - *M.incognita* alone

T3 - *P. aphanidermatum* alone

T4 - *R.solanacearum* alone

T5 - *M. incognita* + *P. aphanidermatum* (simultaneously)

T6 - *M. incognita* + *R. solanacearum* (simultaneously)

T7 - *M. incognita* + *P. aphanidermatum* + *R. solanacearum* (simultaneously)

T8 - *M.incognita* followed by *P.aphanidermatum* (20 days)

T9 - *M.incognita* followed by *R.solanacearum* (20 days)

T10 - *M.incognita* followed by *P.aphanidermatum* + *R. solanacearum* (20 days)

In all the treatments a uniform inoculum of 1000 second-stage juveniles of *M.incognita*/pot was used. In treatments T8, T9 and T10, the plants were inoculated

with nematodes 20 days prior to inoculation of other pathogens. The plants were inoculated with 50 ml. of fungal culture solution and/or 10 ml. of bacterial suspension as the case may be. Appearance of the disease symptoms was recorded daily after inoculation of fungus and bacteria. The experiment was concluded when almost all plants inoculated with fungus/bacterial alone or in combination with nematode expressed disease symptoms.

D. Effect of *M. incognita* and *P. aphanidermatum*

Seed rhizomes of ginger variety, "Himachal" were sown in earthen pots (8" dia.) containing soil mixture (5 1/pot). Three months after germination, 35 pots with uniform plants were selected and inoculated with nematodes (*M. incognita* @ 1000 J2/pot) and fungus (50 ml. of fungal culture solution/pot) alone or in combinations as per the treatments (Table 2) in 5 replications. The pots were arranged in R.B.D. in a net house. Appearance of the disease symptoms was monitored regularly. The experiment was concluded 7 months after sowing. At harvest, observations on growth characterisitcs of ginger and nematode multiplication were recorded.

RESULTS AND DISCUSSION

A. Effect of M. incognita on growth and yield of ginger

The root-knot nematode, *M. incognita* caused significant reduction in the growth and yield of ginger variety "Himachal" (Table 1). Height of the plant (4.32 to 10.30%), weight of roots (7.71 to 29.14%), rhizome (29.60 to 33.35%), total biomass (12.37 to 26.34%) were significantly reduced in plants inoculated with nematodes at different levels. Even at the lowest nematode inoculum level of P=0.2 J2/100 cc. soil, the yield of rhizomes was significantly reduced (29.60%). The economic threshold level of *M. incognita* on ginger was reported as 1 J2/30g soil (0.03 nematodes/g. soil) by Sudha and Sundararaju (1986), 50 J2/100 ml soil (0.5 nematodes/ 1 cc. soil) by Kaur and Sharma (1988) and 2 J2/g soil by Parihar and Yadav (1986). In the present study, even at the lowest inoculum level of 10 nematodes/plant (0.2 nematodes/100 cc. soil) had shown significant effect on the yield of rhizomes. However, number of tillers produced did not vary significantly at any level of nematode inoculum. This may be due to the fact that the plants tend to produce tillers since sufficient moisture was available and these tillers would not have contributed to the yield of rhizomes. Charles and Kuriyan (1980) reported an yield reduction of 46.40% at an inoculum level of 5000 nematodes/ plant (Sudha and Sundararaju, 1986). Colbran (1961; 1962; 1968) and Milne (1979) reported that ginger yields could be substantially increased by controlling the root-knot nematodes through pre-plant soil fumigation. The final nematode populations in the roots did not show any significant differences and were on par in all the plants inoculated with nematodes irrespective of initial inoculum level. This shows that the nematode populations stabilized in due course of time and in this experiment a period of 6 months was sufficient for the nematodes to reach a uniform level in all the treatments. This study also showed that *M.incognita* is a potential pest of ginger and by controlling them the yields can be increased.

B. Effect of *M. incognita, P. aphanidermatum* and *R. solanacearum* alone and in combinations on the incidence of diseases.

The appearance of diseases symptoms caused by the fungus and bacteria were monitored and the data are presented in Fig. 1. The symptom of bacterial wilt caused

by *R. solanacearum* appeared in all the tillers (100%) of the plants inoculated with bacteria alone or in combination with fungus/nematodes expect in treatment T10 where 95.4% tillers showed disease symptoms. In these plants symptoms due to *P. aphanidermatum* could not be observed and bacterial wilt dominated. The symptoms due to fungal infection also appeared from 3rd day onwards after fungal inoculation. The number of tillers showing the disease symptoms was low (16.1%) in the treatment where nematodes and fungus were inoculated with nematodes, 20 days prior to fungal inoculation. In the treatment T5, the disease incidence increased to 44.6% at 7 days and to 100% at 10 days after fungal inoculation, whereas in treatment T8, all the tillers showed disease symptoms by 7th day. In plants inoculated with fungus alone (T3), the symptoms of the diseases (35.4%) appeared only at 7th day and at 10th day 94.0% of the tillers showed diseases symptoms. However, no such disease symptoms could be observed in plants inoculated with nematodes alone (T2) and in control (T1). Samuel and Mathew (1986) reported that root-knot nematode influenced the incidence of bacterial wilt caused by *R. solanacearum*. However, in the present study, root-knot nematode did not show any positive role in the incidence and severity of bacterial wilt of ginger. Further, it was observed that the nematode had a role in the onset and severity of the fungal disease. The severity of the disease was enhanced when plants were inoculated with nematodes prior to fungal inoculation. Similar results were reported by Dohroo et al., (1987).

C. Effect of *M. incognita* and *P. aphanidermatum*

Pot culture test was conducted to assess the precise role of *M. incognita* in the rhizome rot/soft rot of ginger. I was observed that nematode inoculation resulted in the appearance of the disease early in plants inoculated with nematodes and fungus compared to its incidience in plants inoculated with fungus alone. This was more pronounced in plants inoculated with nematodes prior to fungal inoculation (Fig. 2). This may be due to the fact that nematode infested the roots and established before fungal inoculation. The roots already damaged by nematodes might have facilitated the fungus to establish faster and hence the disease appeared faster with more severity in plants inoculated with nematodes prior to fungal inoculation. This study also showed that combined inoculation of nematodes and fungus cuases more damage to ginger. It may also be noted that the yield loss (32.59%) due to *M. incognita* alone in this trial is significant and is comparable with the earlier trial reported in this paper (Table 2). Further, fungus did not alter the nematode multiplication as the final nematode populations in all the treatments with nematodes alone or along with fungus were on par. This further supports the earlier observations on the role of *M. incognita* in the rhizome rot/soft rot caused by *P. aphanidermatum* (Dohroo *et al.*, 1987). In ginger, the main source and spread of root-knot nematodes is infested seed rhizomes and once the infested rhizomes are sown in the field, the nematodes multiply and establish in the field in the early phases of plant growth and the weakened roots are susceptible to pathogens and pests. This is further supported by the results of field experiments where the yields of ginger could be increased by more than 80% by sowing the seed rhizomes treated in hot water in pre-sowing soil fumigated fields (Colbran, 1961; 1962; 1968).

Acknowledgement.

The authors are highly grateful to Dr. K.V. Peter, Director, Indian Institute of Spices Research, Calicut, Kerala for providing necessary facilities and encouragement.

Table 1. Effect of *Melodigoyne incognita* on growth and yield of ginger (Mean of eight replications).

Treatment (J2/100cc soil)	Height (cm)	Shoot weight (kg)	Root weight (kg)	Rhizome weight (kg)	Total biomass (kg)	Nematodes/ g root (final)
0.0	83.62	0.724	0.350	1.439	2.513	-
0.2	78.62 (5.97)	0.828	0.361	1.013 (29.60)	2.202 (12.37)	527.23
2.0	84.50	0.644 (11.04)	0.323 (7.71)	0.959 (33.35)	1.927 (22.31)	478.63
20.0	80.00 (4.32)	0.557 (23.06)	0.296 (15.42)	0.998 (30.64)	1.851 (26.34)	504.66
200.0	75.00 (10.30)	0.612 (15.46)	0.248 (29.14)	1.001 (30.43)	1.862 (25.90)	346.74
$LSD_{0.05}$	6.58	N.S.	0.077	0.327	0.498	N.S.

Table 2. Effect of *Melodigoyne incognita* and *Pythium aphanidermatum* alone and in combination on growth, yield and nematode multiplication in ginger (Mean of five replications).

Treatment	No. tillers	Root weight (kg)	Shoot weight (kg)	Rhizome weight (kg)	Total biomass (kg)	population Final nematode	
						In root (per g)	In soil (per 100cc)
Check	9.25a	0.127a	0.105a	0.270a	0.502a	-	-
Mi alone	7.60ab	0.118a	0.091ab	0.182 b	0.391 b	1806.34 a	123.71a
Py alone	4.60bc	0.035b	0.057b	0.181b	0.273bc	-	-
Mi + Py	8.40 a	0.044 b	0.062 b	0.119 b	0.225 c	2218.29 a	13.84 a
Mi > Py (20d)	6.80 abc	0.071 ab	0.091 ab	0.195 b	0.357 bc	1607.68 a	40.72 a
Mi > Py (40d)	4.00 c	0.084 ab	0.066 b	0.167 b	0.317 bc	1038.01 a	38.51 a
Py > Mi (40d)	8.40 abc	0.045 b	0.065 b	0.127 b	0.237 c	1416.45 a	10.38 a

Means in a column followed by a common letter are not significantly different.

Mi - *Meloidogyne incognita*; Py - *Pythium aphanidermatum*.

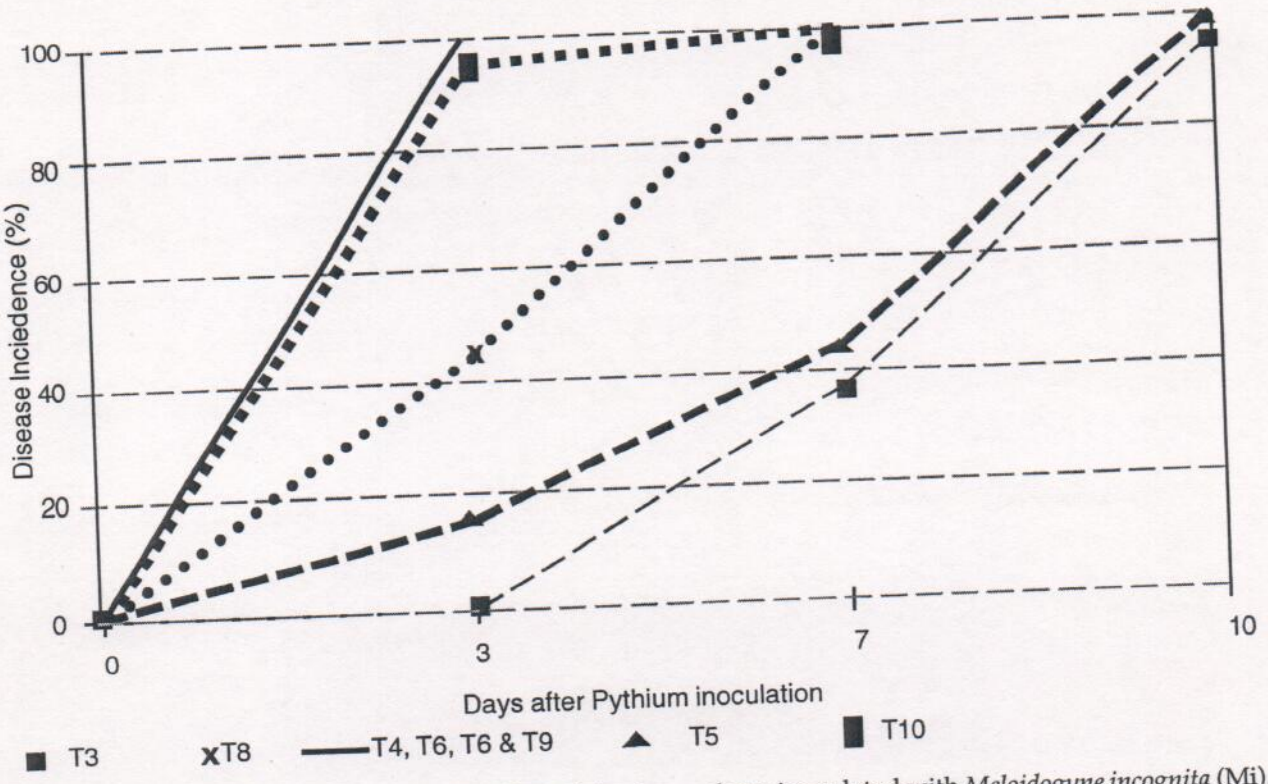


Fig. 1. Disease incidence (per cent tillers affected) in ginger plants inoculated with *Meloidogyne incognita* (Mi), *Pythium aphanidermatum* (Py) and *Ralstonia solanacearum* (Rs) alone and in various combinations (T1 - control, T2- Mi alone, T3 - Py alone, T4- Rs alone, T5 - Mi + Py simultaneously, T6 - Mi + Rs simultaneously, T7 - Mi+Py+ Rs simultaneously, T8- Mi> Py after 20 days, T9 - Mi > Rs after 20 days and T10- Mi > Py+ Rs after 20 days). There was no disease incidence in treatments T1 and T2.

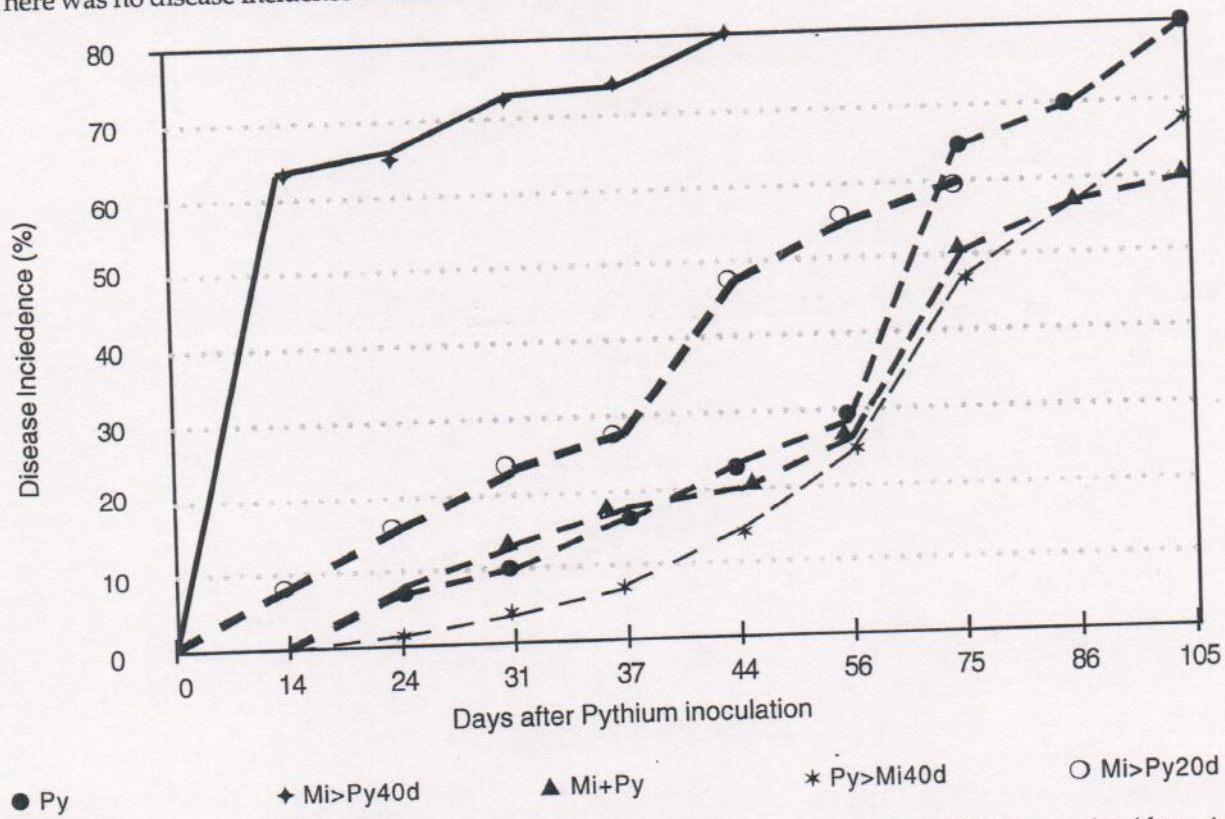


Fig. 2. Incidence of disease in ginger inoculated with *Meloidogyne incognita* and *Pythium aphanidermatum*.

LITERATURE CITED

Charles, J.S. and Kuriyan, K.J. 1980. Studies on nematode incidence in ginger. In: *Proc. PLACROSYM*-II, 1979, pp. 50-57, (Eds) C.S. Venkataram, B. Chandra Mouli, K. Gopalakhrinan, C. Kandasamy, N. Muraleedharan and P. Swaminathan, Indian Society for Plantation Crops, Kasaragod, Kerala.

Colbran, R.C. 1961. Root-knot of ginger. *Qd. agric.* J. 87: 546-547.

Colbran, R.C. 1962. Studies on the root knot nematode control in ginger. *Qd. agric. Sci.* 19: 279-281.

Colbran, R.C. 1968. Nematodes control lifts ginger yields. *Qd. agric.* J. 94: 603-605.

Dohroo, N.P., Shyam, K.R. and Bhardwaj, S.S. 1987. Distribution, diagnosis and incidence of rhizome rot complex of ginger in Himachal Pradesh. *Indian J. Plant Pathol.* 5: 24-25.

Doshi, A. and Mathur, S. 1987. Symptomatology, incidence and management of rhizome rot of ginger by xenobiotics. *Korean J. Plant Prot.*26: 261-265.

Haynes, P.H., Partridge, L.J. and Sivan, P. 1973. Ginger production in Fiji. *Fiji Agric. J.* 35: 51-56.

Huang, C.S. 1966. host parasite relationship of root-knot nematode in edible ginger. *Phytopath.* 5: 24-25.

Kaur, D.J. and Sharma, N.K. 1988. Occurrence and pathogenicity of *Meloidogyne arenaria* on ginger. *Indian Phytopath.* 41: 467-468.

Kulkarni, S.N. and Jani, A.C. 1965. *Meloidogyne* sp. in the rhizomes of sugar (*Zingiber officinale* Rosc.). *Indian Phytopath.* 18: 391-392.

Lanjewar, R.D. and Shukla, V.N. 1985. Parasitism and interactions between *Pythium myriotylum* and *Meloidogyne incognita* in soft rot complex of ginger. *Indian J. Nematol.* 15: 170-173.

Mammen, K.V. 1973. Root gall nematodes as a serious pest of ginger in Kerala. *Curr. Sci.* 42, 549.

Milne, L. 1979. Nematode control on ginger, *Information Bull. Citrus* and *Subtropical Fruit Research Institute* No. 79, pp. 14.

Parihar, A. and Yadav, B.S. 1986. *Meloidogyne incognita* on ginger (*Zingiber officinale* Rosc.) and its control. *Indian J. Mycology and Plant Pathol.* 16: 84-86.

Routaray, B.N., Mohapatra, M.P. and Das, S.N. 1987a. Effect of *Meloidogyne incognita* on ginger, *Zingiber officinale* Rosc. *Indian J. Nematol.* 17: 327-329.

Routaray, B.N. Sahoo, H. and Das, S.N. 1987b. Nemic associations of ginger and turmeric in Orissa. *Indian J. Nematol.* 17: 122-123.

Samuel, M. and Mathew, J. 1986. Role and association of root-knot nematode, *Meloidogyne incognita* in the incidence of bacterial wilt of ginger induced by *Pseudomonas solanacearum. Indian Phytopath.* 36: 398-399.

Sudha, S. and Sundararaju, P. 1986. Pathogenicity of *Meloidogyne incognita* on ginger (Zingiber officinale Rosc.). Indian J. Nematol. 16: 258-259.

Sundararaju, P., Koshy, P.K. and Sosamma, V.K. 1980. Survey of plant parasitic nematodes associated with spices in Kerala and Karanataka. In: Proc. *PLACROSYM II*, 1979, pp. 39-44, (Eds.) C.S. Venkataram, b. Chandra Mouli, K. Gopalakrishnan, C. Kandasamy, N. Muraleedharan and P. Swaminathan, Indian Society for Plantation Crops, Kasaragod, Kerala.

Description of *Meloidogyne piperi* sp.n. (Nematoda: Meloidogynidae) isolated from the roots of *Piper nigrum* in South India

N.K. SAHOO[1], SUDERSHAN GANGULY AND SANTHOSH J. EAPEN[2]

Division of Nematology, Indian Agricultural Research Institute, New Delhi-110012; e mail: sg_nema@iari.ernet.in
[1]Department of Nematology, Orissa University of Agricultural Technology, Bhubaneswar
[2]Indian Institute of Spice Research, Calicut, Kerala-673012

ABSTRACT : One new species of root knot nematode, namely *Meloidogyne piperi* sp.n., collected from the roots of *Piper nigrum* growing in Kerala state of India, has been described and illustrated. The species is diagnosed by the characteristic perineal pattern of its females with broken wavy striae in both dorsal and ventral arch and spreading of ventral striae in lateral region of one side only, numerous striae in perineum region, absence of tail whorl, lateral incisures and posterior protuberance; head region with three annules behind lip cap, the first annule anteriorly directed, the second one low and regressed while the third annule wide and outwardly directed, dosral gland orifice located almost at 4.5 um from the spear knobs, excretory pore located almost opposite to the median bulb, transversely wide spear knobs in females and absence of males. Its second stage juveniles are characterised by having L=310-400 µm; stylet=11-13 µm; dorsal oesophageal gland orifice=3-4 µm; tail=40-53 µm; h=5-16 µm; c=7.5-8.5; c'=5-6, two head annules and distinct rectal dilation. *M. piperi* sp.n. has been differentiated from the closely related species *M. megadora* Whitehead, 1968 and *M. incognita* (Kofoid & White, 1919) Chitwood, 1949.

Key words : *Meloidogyne piperi* sp.n., root-knot nematode, new species, black pepper. *Piper nigrum*, Kerala, India, systematics.

A population of root-knot nematode (*Meloidogyne* sp.) was collected from the roots and rhizosphere of black pepper (*Piper nigrum*) at Calicut, Kerala, India. Its detailed morphological and morphometrical studies were carried out for establishing the species indentity. On comparison of this population with the known species of this genus, it could not be assigned to any of the nominal species and hence being described hereunder as new species.

MATERIALS AND METHODS

The galled roots of black pepper collected form Calicut, Kerala, were stained in acid Fuchsin-lactophenol solution. The mature females were dissected out of the galled tissue and mounted in lactophenol. For some females, perineal sections were prepared and mounted on slides. The egg masses were detached from the roots and then teased for separating the eggs. The eggmassess and mature females were mounted in lactophenol. The unstained eggmasses were incubated for obtaining the second stage juveniles.

The 20 specimens each of mature females, perineal sections, eggs and second stage juveniles (J2), were subjected to the detailed morphological and morphometrical studies. Beside De Man's formula, the other mophometric characters recorded were: distance from head to stylet base (H-St), length and width of median bulb (LMB and WMB), length and width of median valve (LMV and WMV), length and width of neck (L-neck and w-neck), distance from head to medain bulb (H-MB), for mature females; length of vulval slit (LVS), distance from anus to vulval slit (AVS), anus to tail terminus (ATT) and inter phasmidial distance (IPD) for perineal patterns; length (L), width (W) and ratio a for eggs; distance from head to median bulb (H-MB), median valve (H-MV), excretory pore (H-Excp.), and width (ABW), hyaline part of tail (h) for second stage juveniles. All observations were made on Research Microscope Olympus BX 50 and the camera-lucida drawings were made using the drawing-tube attachment. The arithmetic mean and standard deviation for each measurement were

Part of Ph.D. thesis submitted by the senior author the the P.G. School, I.A.R.I., New Delhi-110012

calculated. The data thus obtained was compared with the known species *Meloidogyne*.

MELOIDOGYNE PIPERI SP. N.

(Fig. 1 & 2; Plate I)

Measurements

Paratype females (n=20): L = 720µm ± 45 (555-874), W =533µm ± 43 (400-685), Stylet = 14.3µm ± 0.5 (13-16), H-St = 16.4µm ± 0.8 (14-20), DGO = 4.5µm ± 0.3 (4-6), Neck = 154µm ± 13.6 (120-207), W-neck = 36µm ± 2.8 (70-200), LMB = 38µm ± 2.1 (30-45), WMB =36µm ± 2.8 (30-50), LMV =12µm ± 03 (12-14), WMC = 11.2µm ± 0.6 (9-13), H- Ex = 33-64, a = 1.4 ± 0.1 (1.1-1.6) H-MB = 50µm ± (30-70), b_1 = 15.4 ± 2.3 (7.9-26.8), a (neck) = 1.2 ± 0.2 (0.7-1.8), vulval slit length = 19.9µm ± 1.3 (18-22), Anus to vulval slit distance = 13.3µm ± 0.8 (12-14.5), anus to tail terminus distance = 10.8 ± 1 (10-12), Inter phasmidial distance = 19µm ± 1.9 (1.9 (15-22).

Holotype (female); L = 780µm, W = 515µm, Sylet = 13µm, DGO = 4µm, LMB = 32µm, WMB = 28.5µm, LMV = 12µm, WMV = 9.5µm, H-Exp = 57µm, a = 1.5

Eggs: (n=20) : Length = 85µm ± 2.4 (77-92), width = 37µm ± 1.1 (33-42), 'a' ratio = 2.3 ± 0.1 (2.1-2.7).

Second stage juveniles (n=20) : Length = 366µm ± 15 (310-400), maximum body width = 12.9µm ± 0.4 (12-14), stylet = 12.2µm ± 0.3 (11-13), H-st = 12.9µm ±0.2 (12-13.5), DGO = 3.6µm ± 0.2 (3-4), LMB = 12.6µm ± 0.4 (11-14), WMB = 7.2µm ± 0.3 (6-8), H-MB = 45µm ± 1.7 (40-50), H-MV = 51µm ± 1.8 (46-56), H-Oij = 74µm ± 27 (62-80), H-O =147µm ± 7.0 (123-168), H-Exp = 74µm ± 2.8 (62-81), Tail = 47µm ± 1.9 (40-53), h= 8.4µm ± 1.5 (5-16), ABW = 8.6µm ± 0.4 (7.5-10), a = 28.4 ± 1.1 (26-33), b = 4.9 ± 0.1 (4.3-5.4), b' = 2.5 ± 0.2 (1.9-3.1) b_1 = 8.2 0.3 (7.3 -9.1), c= 7.8 ± 0.2 (7.5-8.5), c' = 5.5 ± 0.1 (5.1-6), O% = 29 ± 1.4 (24-32).

Description

Mature female: Body opaque, pyriform with short neck (Fig 2; plate I). In a few specimens, the dorsal curvature of the body was more than the ventral. Posterior protuberance absent. Lip cap present. Head with three annules, the first annule being anteriorly directed, the second one low and regressed while third annule wide and outwardly directed. Stylet slender with its dorsally curved conus. Stylet knobs short. Excretory pore located about 2 stylet lengths poaterior to spear knobs almost at the level of median bulb.

Perineal pattern: The perineal pattern oval with high dorsal arch in most of the specimens, having broken wavy striae in both dorsal and ventral arches (Fig. 1, A-E Plate I). Lateral field and lateral lines absent. The lateral forking of a few striations of the ventral arch seen near the lateral region on one side. Broken striae in the ventral arch directed toward right lateral region in one side only and sometimes spreading up to the middle of dorsal arch. Also, broken striae spread toward the perineum making the perineum region very narrow. Tail whorl absent, phasmids closely spaced located near the tail tip.

Second stage juveniles (J_2) : Body slender, tapering at both the ends. Head hemispherical slightly set off from body having a lip cap followed by two head annules. Basal plate thin. Dorsal gland orifice located more than one fourth of the stylet length behind the spear base, cephalids absent, hemizonid 3 annules long. Excretory pore located 8-9 annules posterior to hemizonid. Oesophageal glands overlapping the intestine ventrally, lateral field with four incisures, phasmids located at the mid region of tail, rectum inflated, tail tapers with a notch just posterior to hyaline region and ends with subacute termins.In an aberrant form tail narrows abruptly below the hyaline region forming a distinct notch and then ending in a digitate tail terminus (Fig 2,E).

Male : Note found.

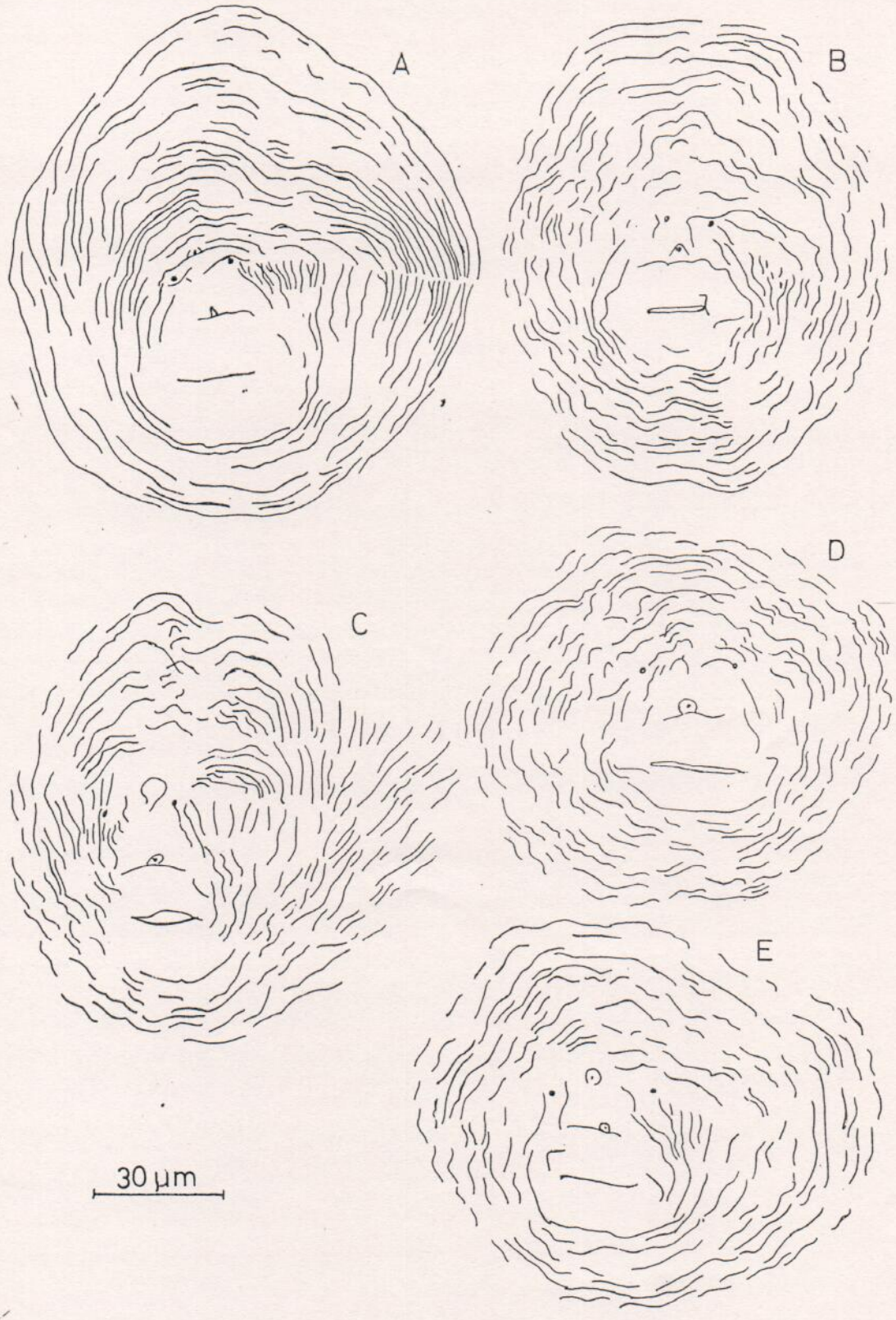


Fig. 1. (A-E). *Meloidogyne piperi* sp.n., variation in perineal patterns of females

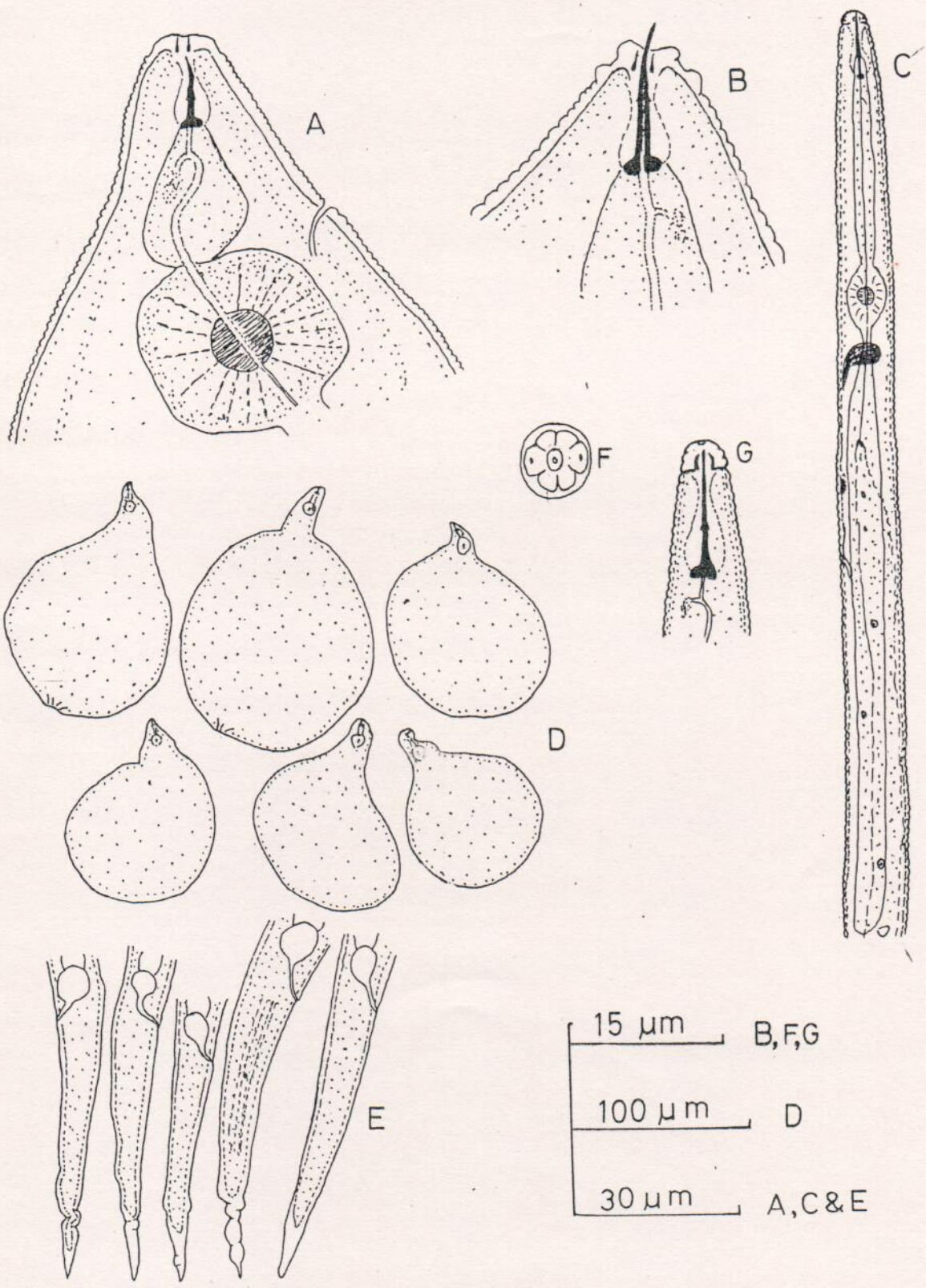
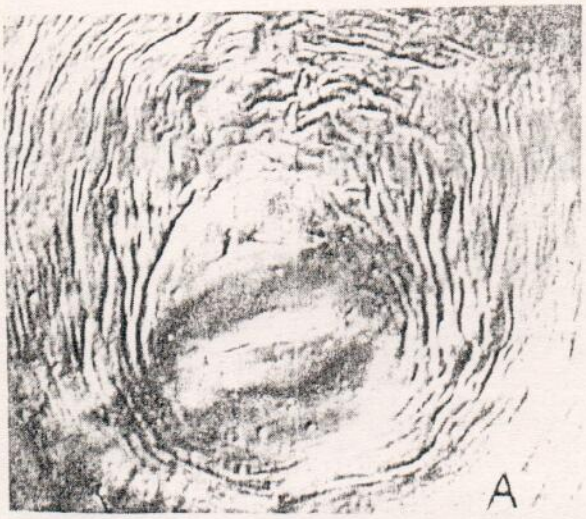
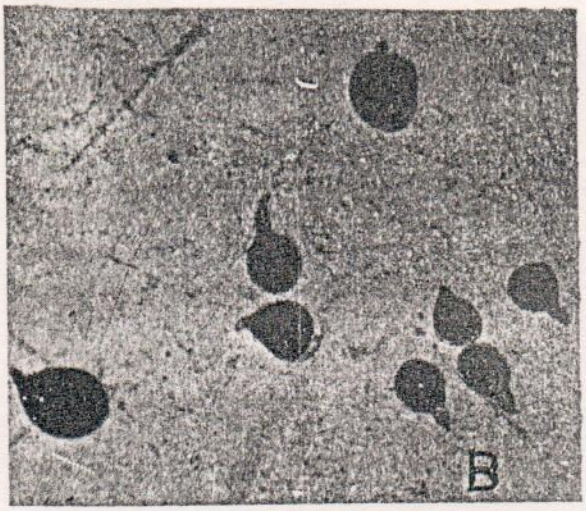


Fig. 2 (A-G). ***Meloidogyne piperi*** **sp.n. A, B, D & F : Female; C, E & G : Second stage juveniles; A, B, C & G : Anterior regions; D: Entire females; E: Tail regions; F :** ***Enface*** **view.**





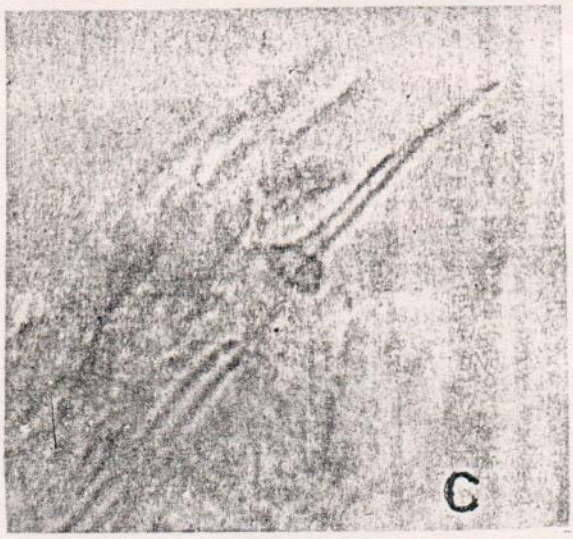


Plate I (A-C). ***Meloidogyne piperi*** **sp.n., females. A : Perineal pattern; B : Entire females C : Excised stylet.**

Diagnosis and relationships : *Meloidogyne piperi* sp.n. is diagnosed by the characteristic perineal pattern of the female with broken wavy striae in both dorsal and ventral arch and spreading of ventral striae in lateral region of one side only, and absence of posterior protuberance; lip region with three annules behind lip cap, the first annule anteriorly directed, the second one low and regressed while the third annule wide and outwardly directed, in females. The new species have been compared with two closely related species ie. *M. megadora* Whitehead, 1968 and *M. incognita* (Kofoid & White, 1919) Chitwood, 1949, the selected differentiating characters being outlined in Table 1.

M. piperi sp.n. resembles with *M. megadora* Whitehead, 1968 in shape of female body, stylet and tail of J_2, and spreading of striae of the ventral arch towards lateral region in perineal pattern. However, it differs from this species in shape of perineal pattern, stylet knobs and the position of excretory pore in female, and smaller size of second stage juveniles. In new species, the dorsal arch is higher than the ventral with many broken striae in both the arches; and the broken striae concentrate toward the perineum region thus making this region very narrow. (In *M. megadora*, the dorsal arch is low as compared to ventral arch and overall perineal pattern is circular, no lateral spreading of the striae; and J_2=413-458 µm). The position of excretory pore in female of *M. megadora* is about the level of stylet knobs but in the new species its position is far posterior to the stylet knobs almost opposite to the median bulb. Moreover, in *M. megadora* spear knobs are posteriorly sloping in female whereas transversely wide in the new species.

This species also resembles with *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 due to its high dorsal arch of the perineal pattern with broken striae and forking at the lateral field, shape of the head of mature female and tail length of J_2, but differs in perineal pattern wherein striae of the ventral arch spread toward lateral region and even in some patterns the spreading of striae proceed to the

Table 1. Comparison of selected taxonomic characters of females and second stage juveniles of *Meloidogyne piperi* sp.n. with the paratype measurements of closely related species.

Species	Females							Second stage juveniles							
	L (µm)	W (µm)	Stylet (µm)	Stylet knobs	Dgo (µm)	Excp to stylet	Post. Prot.	L (µm)	c	Stylet (µm)	dgo (µm)	Hem to Excp.	Rect. dil.	Tail (µm)	H (µm)
M. megadora	683 (554-845)	471	15 (13-17)	PS	?	even	P/A	413-458	8-11	11-13	?	Ant.	A	53 (47-58)	6±6
M. incognita	510-690	300-430	16	AI	3*	1 style post.	A	360-393	8-9	10	2-2.3	Ant. but adj.*	MP*	49 (45-52)	9±2 (6-14)
M. piperi sp.n.	720 (555-874)	533; 400-685	14 (13-16)	TW	4.5 (4-6)	2stylet post.	A	310-400	7.5-8.5 8.5)	11-13	3-4	Ant.	P	47+1.9 (40-53)	8±1.5 (5-16)

L=body length; W=body width; dgo= position of dorsal oesophageal gland orifice; Stylet knobs (PS-posterioly sloping; AI=Anteriorly indented; TW= transversely wide); Excp. to stylet = excretory pore in relation to stylet (even=opposite to the knobs; 1stylet post. =1stylet length posterior to the knobs; 2 stylet post = 2stylet lengths posterior to the knobs); Post. prot. =posterior protuberance (P=present; A=absent); Hem. to excp= hemizonid in relation to excretory pre (Ant. = anterior ; adj = adjacent); Rect. dil. = rectal dilation (P=present; A = absent; MP = mostly present); h=hyaline part of the tail.

*** As seen in Indian populations by the authors.**

middle of dorsal arch; also concentration of striae around perineum making the perineum region narrow. In *M. incognita*, there is no spreading of ventral arch striae in lateral region, and not many striae in perinuem region. *M. piperi* sp.n. further differs from *M. incognita* in having transversely wide rounded spear knobs in females, DGO located more posteriorly at 4-6µm and 3-4µm behind the spear knobs in females and juveniles respectively, excretory pore located posteriorly at the level of median bulb, bigger size of females (720 x 533 µm) and eggs (85 x 37 µm). While in *M. incognita*, the spear knobs in females are indented anteriorly; DGO located at 3 µm and 2-2.5 µm behind spear knobs in female and juveniles, respectively the excretory pore in females located anteriorly almost at the level of spear knobs and the size of females and eggs is smaller i.e. 609 x 415 µm and 77 x 32 µm, respectively.

Type host and locality : Specimens collected from the roots of black pepper (*Piper nigrum*) at Calicut, Kerala.

Other host : Brinjal (*Solanum melongena*).

Type deposition : Holotype female, 20 paratype females, 5 paratype perineal patterns and 20 paratype second stage juveniles on slides deposited in National Nematode Collection of India at Division of Nematology, IARI, New Delhi - 110 012, vide accession nos. 1948 to 1955.

Collector's name : Dr. Santhosh J. Eapen.

Etymology : Type species is named '*piperi*' after its type host *Piper nigrum*.

REFERENCES

Chitwood, B.G. (1949). Root-knot nematodes. Part-I. A revision of the genus *Meloidogyne* Goeldi, 1887. *Proc. Helminth. Soc. Wash.* **16** : 90-104.

Kofoid, C.A. & White, W.A. (1919). A new nematode infection in man. *J. Am. Med. Ass.* **72** : 567-569.

Whitehead, A.G. (1968). Taxonomy of *Meloidogyne* (Nematoda : Heteroderidae) with descriptions of four new species. *Trans. Zool. Soc.*, London. **31** : 263-401.

Accepted for publication : November, 2000