**CONTENTS**

## PART I: GENERAL INFORMATION

**800 Project Code**

8001 Institute Project Code No. : Biocontrol 1.3 (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 : BIOLOGICAL CONTROL OF NEMATODES IN SPICES (Formerly 'Biological Control of Pests and Diseases of Spices')**

**803 Priority Area** :

8031 Research Approach :

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

**804 Specific Area** : **BIOLOGICAL CONTROL**

**805 Duration of Project** :

8051 Date of start : 1992

8052 Date of Completion : 2005

**806 Total cost /Expenditure Incurred** : Rs. 16.21 lakhs

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

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

## 807 EXECUTIVE SUMMARY

Several fungi and bacteria were isolated from soil samples collected from healthy black pepper, ginger, cardamom and turmeric plants in sick plots of various spice growing states. Similarly microorganisms from the rhizosphere of nematode antagonistic plants were also isolated. Three isolates of *Pasteuria penetrans* were collected from ginger soil samples obtained from Sikkim, Wynad and Kasargod. Besides a number of fungi and bacteria available in the IISR repository were also studied for their efficacy to suppress major plant parasitic nematodes of spices.

New methods of laboratory screening were standardized for in vitro screening of fungi and bacteria against nematodes. Egg parasitic fungal isolates of *Pochonia* (= *Verticillium*) spp., *Paecilomyces lilacinus, Trichoderma* spp., *Fusarium* spp., *Aspergillus* spp., and the obligate bacterial hyper parasite *Pasteuria* spp., were identified through *in vitro* bioassays. Several rhizobacteria showed significant suppression of root knot nematodes under *in vitro* conditions.

Inoculation of the biocontrol agent, *P. penetrans*, simultaneously with root knot nematodes or 15 days after nematode inoculation significantly reduced the total biomass of cardamom plants. *Pochonia chlamydosporia* partly suppressed *R. similis* population in black pepper rooted cuttings. *P. chlamydosporia, P. lilacinus, Fusarium* sp., *Aspergillus nidulans* and *Scopuloriopsis* sp. are the best anatagonsitic fungi that suppressed root knot nematode populations significantly.

Five isolates of rhizobacteria (IISR 522, IISR 528, IISR 658, IISR 853 and IISR 859) having dual nematicidal action (suppressing both *R. similis* and *M. incognita*) were short-listed from a collection of 291 isolates. IISR 658, IISR 853 and IISR 869 inhibited *P. capsici* in laboratory tests.

Both *P. chlamydosporia* and *P. penetrans* improved the growth of cardamom seedlings by suppressing root knot nematodes under field conditions. In a farmer's plot at Wynad, *T. harzianum, P. chlamydosporia* and *P. penetrans* significant reduced nematode populations. *P. chlamydosporia, Fusarium* sp. and *Scopulariopsis* sp. also significantly increased the yield of ginger besides controlling nematodes.

Field application of IISR 522, IISR 528, IISR 658, IISR 853 or IISR 859, promising rhizobacteria, reduced the foliar yellowing in black pepper vines and suppressed plant parasitic nematodes. Application of IISR 13, IISR 51, of IISR 522, IISR 658 and IISR 866 in ginger field also increased the yield by 31.8 – 56.4 %. However in turmeric, IISR 853 followed by LS.151 and IISR 6 gave the maximum yield.

Sorghum and decomposed coffee husk supported maximum growth and sporulation of *P. chlamydosporia*, while FYM and vermicompost were the least preferred substrates. But maximum growth and multiplication of *P. lilacinus* were observed in rice and ginger leaf powder. The optimum pH and temperature conditions for growth and multiplication of *P. chlamydosporia* were determined to be $5^0$C and $26^0$C, respectively. The fungus multiplied well in starch water and coconut water also.

Genomic DNA of nematicidal rhizobacteria isolated from black pepper, cardamom, ginger etc. was isolated through the CTAB-SDS method and procedure for ARDRA (Amplified Ribosomal DNA Restriction Analysis) finger printing was standardized. Molecular characterization of 39 isolates of rhizobacteria, diverse in their morphology, phenotypic characters, antibiotic resistance and their antagonistic activity, was completed through ARDRA and rep-PCR. Variability in *P. chlamydosporia* and related was also studied.

**808 Key words :**
Biological control, *Pochonia chlamydosporia, Pasteuria penetrans,* root knot nematodes, Amplified Ribosomal DNA Restriction Analysis, rhizobacteria, secondary metabolites, *Trichoderma harzianum, Paecilomyces lilacinus*

## PART II : INVESTGATORS' PROFILE

**810 Principal Investigator :**

| | | | |
|---|---|---|---|
| 81011 | Name | : | **M. Anandaraj** (1992-94) |
| 81021 | Designation | : | Scientist SG (Plant Pathology) |
| 81012 | Name | : | **Santhosh J. Eapen** (1994-2006) |
| 81022 | Designation | : | Sr. Scientist (Nematology) |
| 8103 | Division/ Section | : | Division of Crop Protection |
| 8104 | Location | : | IISR, Calicut |
| 8105 | Institute Address | : | P.B. No. 1701, Marikunnu Post<br>Calicut – 673 012, Kerala |

**811 Co- Investigators:**

| | | | |
|---|---|---|---|
| 81111 | Name | : | Santhosh J. Eapen (1992-94) |
| 81121 | Designation | : | Scientist (Nematology) |
| 81112 | Name | : | Y. R. Sarma (1992-97) |
| 81122 | Designation | : | Principal Scientist & Head |
| 81113 | Name | : | K.V. Ramana (1994-02) |
| 81123 | Designation | : | Principal Scientist |
| 81114 | Name | : | M. Anandaraj (1995-96) |
| 81124 | Designation | : | Scientist SG (Plant Pathology) |
| 81115 | Name | : | M. N. Venugopal (1992-94) |
| 81125 | Designation | : | Sr. Scientist (Plant Pathology) |
| 81116 | Name | : | A Kumar (1997-06) |
| 81126 | Designation | : | Scientist SS |
| 8113 | Division/ Section | : | Division of Crop Protection |
| 8114 | Location | : | IISR, Calicut |
| 8115 | Institute Address | : | P.B. No. 1701, Marikunnu Post<br>Calicut – 673 012, Kerala |

## PART III: TECHNICAL DETAILS

### 820 Introduction and objectives

8201 Project objectives

- To isolate and identify native microorganisms antagonistic to plant parasitic nematodes infesting spices.
- To develop a nematode biocontrol agent to be used as a component of integrated nematode management in spices

8202 Background information and importance of the project

Spice crops are affected by a multitude of diseases, nematodes and insect pests which are major production constraints. *Phytophthora* foot rot and slow decline of black pepper, clump rot and capsule rot of cardamom, rhizome rot of ginger and turmeric are major diseases. Burrowing nematodes (*Radopholus similis*) and root knot nematode (*Meloidogyne incognita*) are the most important nematode pests of black pepper. Root knot nematodes attack other major spice crops like cardamom, ginger and turmeric.

In spices there is an urgent need for the development of a non-chemical and eco-friendly control measures as chemical nematicides are still the primary option for managing nematodes. Such work is necessary to develop environmentally sound agricultural systems that minimize chemical use while maintaining high production standards. The tropical soils are rich in biodiversity of beneficial microbes and the biocontrol potential of the resident microbial fauna and flora is under exploited. Suppression of plant-parasitic nematodes with nematode predators, parasites or disease agents is a desirable alternative to chemicals. This emerging area is called biological control. In a nematological perspective, biological control is the reduction of nematode populations, which is accomplished through the action of living organisms other than nematode-resistant host plants, which occur naturally or through the manipulation of the environment or the introduction of antagonists. The mechanisms involved in biological control of nematodes are mainly hyper-parasitism, predation, competition and antibiosis.

Hundreds of organisms, which parasitize or prey on nematodes, are reported and they belong to diverse taxa including nematode trapping or endoparasitic fungi, predatory nematodes, arthropods (e.g. mites and collembola), bacterial parasites, and predatory protozoa. Deploying and managing biocontrol agents will likely become increasingly important components of integrated pest

management programs and sustainable agricultural systems. Therefore, this project aims at identification of potential biological control agents, developing techniques for their mass multiplication in the laboratory and use as a component in integrated pest management programs in the field.

**821 Project Technical Profile**

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

- Isolation and identification of antagonists of nematodes from the rhizosphere and their identification
- Testing the efficiency of potential biocontrol agents (BCAs) *in vitro* and *in vivo*.
- Standardizing mass multiplication techniques of promising BCAs.
- Evaluation of promising BCAs in the field.
- Studies on mode of action of BCAs against nematodes.
- Studies on characterization of promising isolates.

8212 Total man months involvement of component project workers

81 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

The project has helped to develop non-chemical means of managing plant parasitic nematodes of spices wherein native isolates of *P. chlamydosporia, T. harzianum, P. penetrans,* rhizobacteria etc. can be effectively used on a large scale for control of nematodes infesting spices.

8223 Process/ Product/ Technology/ Developed

- Incorporation of *Trichoderma harzianum* multiplied on decomposed coffee husks (7 days old) at the time of sowing @ 2.5 kg / bed (4.5 m x 1 m) and after three months for control of root knot nematodes and damping off in nurseries.
- Application of *Pochonia chlamydosporia* (*Verticillium chlamydosporium*) multiplied on rice grains or sorghum @ 50g /vine twice a year during April/May and September/October.

- Fortifying black pepper nursery mixture with biocontrol agents like *T. harzianum*, *P. lilacinus*, *P. chlamydosporia* and rhizobacteria.

8225 Practical Utility
(not more than 150 words)

Spices are an important commodity of foreign exchange that contributes to Indian economy. India is traditionally considered as the 'land of spices'. But productivity of many spices in India is very low mainly because of the damage caused by pests and pathogens. Effective chemical control methods against major pests and pathogens of spices are available. However, prolonged use of chemical is not desirable as the pesticide residues pollute the atmosphere and cause health hazards. To evolve a viable technology involving reduced use of pesticides, Integrated Pest Management (IPM) strategies are necessary. The results obtained in this project would be useful in developing IPM strategies, which would minimize the use of chemicals and would promote eco-friendly nematode management. This is highly relevant and readily applied in organic agriculture for which there is a global awareness.

8225 Constraints, if any

- Non availability of biosafety data for any of these new bioagents
- Lack of entrepreneurship in commercialization of these technologies
- Inadequate manpower for in-house mass multiplication of bioagents
- Poor awareness among the growers regarding the need for nematode control

## 823 PUBLICATIONS AND MATERIAL DEVELOPMENT

8231 Reviews

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

b. 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. (Eds.) G. Swarup, D.R. Dasgupta & J.S. Gill. Nematological Society of India, New Delhi, India.

c. Sarma Y.R., Ramana K.V., Dake, G.N., Venugopal, M.N., Anandaraj M., Devasahayam S and Eapen S.J. 1996. Biocontrol strategies in pest and disease management of spice crops (Abstract). *2nd International Crop Science Congress*, p.336. Nov. 17-24, 1996. National Academy of Agricultural Sciences and Indian Council of Agricultural Research, New Delhi.

d. Sarma Y.R., Ramana K.V., Dake, G.N., Venugopal, M.N., Anandaraj M., Devasahayam S and Eapen S.J. 1996. Biological control of pests and diseases (Abstract). *National Seminar on Biotechnology of Spices and Aromatic Plants*, 24-25 April 1996. Calicut, India.

e. Santhosh J Eapen and K V Ramana. 1997 Biological control of plant parasitic nematodes. In: *Status of Biological Control in Spices*, M. Anandaraj and K V Peter (Eds.),pp.20-32, Indian Institute of Spices Research, Calicut, Kerala, India.

f. Santhosh J Eapen, Ramana K V and Y R Sarma. 1997. Biological control of plant parasitic nematodes of major spice crops: Present status and future (Abstract). *Symposium on 'Economically important diseases of crop plants'*. 18-20 December 1997, Indian Phytopathological Society (Southern Chapter), Bangalore, India. p.59.

g. 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.

h. 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.

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

j. 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.

k. Santhosh J Eapen and Ramana K V 2000. *Verticillium chlamydosporium*, a nematophagous fungus for the management of nematodes of spices. *International Conference on Plantation Crops*. December 12-15, 2000.

l. 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.

m. 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.

n. 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.

o. 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).

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

8232 Research papers

a. Sreeja T.P., Eapen S.J and Ramana K.V. 1996. Occurrence of *Verticillium chlamydosporium* Goddard in a black pepper (*piper nigrum* L.) garden in Kerala, India. *J. Spices & Aromatic Crops* 5: 143 – 147.

b. Santhosh J Eapen, Ramana K V. and Y R Sarma. 1997. Evaluation of *Pseudomonas fluorescens* isolates for control of *Meloidogyne incognita* in black pepper (Piper nigrum L.). in: *Biotechnology of Spices*, *Medicinal and Aromatic Plants*, pp. 129-137 (Eds.) S. Edison *et al* ., Indian Society for Spices, Calicut, India.

c. Santhosh J Eapen, Venugopal M N, Ramana K V and Sarma Y R. 2000. *Trichoderma* spp. for the management of root knot nematodes and rhizome rot disease in cardamom nurseries. *In: Recent Advances in Plantation Crops Research*, (Eds.) N. Muraleedharan & R. Rajkumar, pp. 382 – 386. Allied Publishers Ltd., Mumbai, India.

d. Santhosh J Eapen, Beena. B and Ramana K V. 2000. Preliminary screening of some hyphomycetous fugi for the biological control of root-knot nematodes (*Meloidogyne incognita*) infesting spice crops. *National Nematology Symposium on integrated Nematode Management*. November 23-24, 2000. Orissa University of Agriculture & Technology, Bhubaneswar, Orissa. P 18.

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g. Beena B., Santhosh J. Eapen and Ramana, K.V. 2003. Native

rhizobacteria for the biological suppression of *Radopholus similis* infesting black pepper (*Piper nigrum* L.). *6th International PGPR Workshop*, 5-10 October 2003, Indian Institute of Spices Research, Calicut. pp. 12.

h. Santhosh J. Eapen, B. Beena and K.V. Ramana 2005. Tropical soil microflora of spice-based cropping systems as potential antagonists of root-knot nematodes. *Journal of Invertebrate Pathology* 88: 212 – 220. (Elsevier).

i. Kumar, A., Dinu, A., Aravind, R., Eapen, S.J., Jisha, S., Anila, G., Beena, N., Anandaraj, M. and K.V.Ramana 2004. Evaluation of genetic diversity of rhizobacterial strains obtained from spice crops. *Agri-Informatics 2004*, pp. 49-55, Indian Institute of Spices Research, Calicut.

j. Bhai, R.S., Kishore, V.K., Kumar, A., Anandaraj, M. and Eapen, S.J. 2005. Screening of rhizobacterial isolates against soft rot disease of ginger (Zingiber officinale Rosc.). *Journal of Spices and Aromatic Crops* 14: 130-136.

8233 Popular articles

a. Sarma, Y.R., Anandaraj, M., Venugopal, M.N., Suseela Bai, 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. 'Biocontrol agents in organic farming'. *Spice India* (Malayalam) 12: 12-13, 22-23.

c. Ramana K V, Santhosh J Eapen, 2000. Biocontrol of nematodes in spice crops. *ICAR News* 6(4): 16-18.

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a. Jissa N. Varghese 2002. Standardization of Optimum Conditions for Mass Multiplication of *Verticillium chlamydosporium*, a Potential Nematode Biocontrol Agent. M.Sc. Project Report. Bharathidasan University, Thiruchirappalli, Tamil Nadu.

b. Mini Mani. 2002. Effect of Copper Oxychloride, Different Sources of Carbon and Nitrogen on the Growth of *Verticillium* spp. M.Sc. Project Report. Bharathiar University, Coimbatore, Tamil Nadu.

c. Shybi Sebastian 2002. Evaluation of Nitrogen Fixing and Phosphate Solubilizing Bacteria for the Control of Root Knot Nematode (*Meloidogyne incognita*). M.Sc. Project Report. Bharathidasan University, Thiruchirappalli, Tamil Nadu.

d. Manju S. 2003. Mass multiplication of *Verticillium chlamydosporium* on plant extracts and plant based solid substrates. M.Sc. Project Report submitted to Bharatidasan University, Tiruchirappally, Tamil Nadu.

e. Britto Cathrin B. 2004. Comparative genomics of biocontrol genes present in five species of Pseudomonas. M.Sc. Project Report submitted to Bharatidasan University, Tiruchirappally, Tamil Nadu.

f. Sreesmitha, V. 2005. Designing of species specific primers for plant growth promoting rhizobacteria (PGPR), *Bacillus* and *Pseudomonas*

based on 16S rDNA polymorphism. M.Sc. Project Report submitted to Periyar Universit, Tamil Nadu.

8235 Seminars, conferences and workshops (relevant to the project) in which the scientists have participated. (List abstracts forwarded)

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

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

c. Sixth Biocontrol Workers Group Meeting, Project Directorate of Biological Control, Bangalore, Karnataka, 19-20 June 1997.

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

e. 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

s. 6$^{th}$ International PGPR Workshop, 5-10 October 2003, Indian Institute of Spices Research, Calicut.

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

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

8241 Infrastructure developed

- *Biocontrol Laboratory* – A well established laboratory for isolation, characterization and in vitro testing of biocontrol agents.
- Facilities like laminar flow unit, image analyzing system, digital photography etc.

8242 Details of field, laboratory note books

Laboratory/field note books – 3 Nos. (Available with Senior Scientist, Nematology)

Data files - 2 Nos. (Available with Senior Scientist, Nematology)

8243 Biocontrol organisms

All the promising cultures have been deposited in the IISR repository (IISR 1560 to IISR 1576).

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

A number of potential nematode antagonists have been identified through this study. Since the field efficacy of some of them is proved beyond any doubt, efforts should be made for their popularization. These technologies can be commercialized provided biosafety data is generated for them.

**PART IV: PROJECT EXPENDITURE** (Summary)

**Year 1992-2005**

**830 Total Recurring Expenditure**

8301 Salaries: (Designation with pay scale)

| | | Estimated (Rs.) | Actual (Rs.) |
|---|---|---|---|
| i) Scientific | | 4,00,000 | 10,25,000 |
| ii) Technical | | 50,000 | 1,10,000 |
| iii) Supporting | | 20,000 | 1,14,000 |
| iv) Wages | | 10,000 | 57,000 |
| | Sub-Total | 4,80,000 | 13,06,000 |

8302 Consumables

| | | | |
|---|---|---|---|
| i) Chemicals | | 20,000 | 77,000 |
| ii) Glasswares | | - | 53,000 |
| iii) Others | | - | 51,000 |
| | Sub-Total | 20,000 | 1,81,000 |

| | | | |
|---|---|---|---|
| 8303 | Travel | - | 28,000 |
| 8304 | Miscellaneous (other costs) | - | 61,000 |
| 8305 | Sub-Total (Recurring) | 5,00,000 | 15,76,000 |

**831 Total Non – Recurring** Expenditure (Equipments and works)

| | | |
|---|---|---|
| i) Laminar flow unit | 1 No. | 45,000 |

| | | | |
|---|---|---|---|
| **832** | **Total** (830 and 831) | 5,00,000 | 16,21,000 |

## 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.

2.

Signature & Comments of the Head
Of the Division/ Section

Signature & Comments of the
Joint Director (Research)

Signature & Comments of the
Director

# ANNEXURE I

## ISOLATION AND IDENTIFICATION OF MICROORGANISMS

Soil samples from the rhizosphere of black pepper, ginger, turmeric and cardamom were collected from spice based agro-ecosystems in Kerala, Karnataka and Tamil Nadu. Fungi and bacteria present in these soils were isolated using standard procedures. They were cultured and maintained for further studies. Altogether 57 bacteria and 73 fungi were isolated (Table 1 and Fig. 1 & 2). Out of the 73 fungi, 61 isolates were saprophytes, some of which were previously reported as facultative parasites of nematodes. Majority of them (17 isolates) belonged to the genus *Trichoderma*, while 7 isolates were of *Aspergillus* spp., 6 each of *Paecilomyces* spp. and *Pencillium* spp., 5 isolates of *Fusarium* spp., and one each isolate of *Pochonia chlamydosporia* and *Verticillium lecanii*. Taxonomic identity of 22 isolates could not be established as there was no sporulation. *P. chlamydosporia* Goddard, a known biocontrol agent against cyst and root knot nematodes, was isolated for the first time from cases of *Trophotyenchulus piperis* infesting black pepper at the District Agricultural Farm, Koothali, Calicut District. The association of this fungus with roots of black pepper was also noticed.

Among the 57 bacteria isolated, 17 isolates belonged to different species of *Bacillus* while 12 were *Pseudomonas fluorescens* isolates. Three isolates of *Pasteuria penetrans* were obtained from ginger samples collected from Kasaragod District. About 50 per cent of the bacterial isolates could not be identified even up to the generic level as they lacked easily distinguishable taxonomic features.

Earlier studies in spice agro ecosystems showed the prevalence of these fungi in the rhizosphere soil of spices like black pepper and cardamom (Sankaran, 1981). That is why rhizosphere is considered as the first line of defense for roots against attack by soil-borne pathogens (Weller, 1988). Fungi like *A. niger*, *F. oxysporum* and *P. lilacinus* were frequently isolated from egg masses of root-knot nematodes by other workers too (Goswami *et al*., 1998a). Some fungi, such as *P. chlamydosporia*, are largely confined to the rhizosphere. Many of the bacteria obtained in this study could not be identified even up to the genus level by the common procedures. However, isolates of *Bacillus* spp. and *P. fluorescens*, two groups of rhizobacteria that are known for their nematotoxic properties, could be distinguished from the rest. Similar results have been reported with other rhizobacteria and parasitic nematodes (Becker *et al*., 1988; Oostendorp & Sikora, 1989; Racke & Sikora, 1992).

Table 1. ***Bacteria and fungi isolated from the rhizosphere of spices.***

| Antagonist | Black pepper | Cardamom | Ginger | TOTAL |
|---|---|---|---|---|
| **BACTERIA** | | | | |
| *Bacillus* spp. | 5 | - | 12 | 17 |
| *Pseudomonas flourescens* | 8 | - | 4 | 12 |
| *Pasteuria* sp. | - | - | 3 | 3 |
| Unidentified | 12 | - | 13 | 25 |
| **FUNGI** | | | | |
| *Aspergillus* sp. | 1 | - | - | 1 |
| *A. fumigatus* | 1 | - | 1 | 2 |
| *A. nidulans* | - | - | 1 | 1 |
| *A. restrictus* | - | - | 1 | 1 |
| *A. tamarii* | - | - | 1 | 1 |
| *A.ustus* | - | - | 1 | 1 |
| *Aurobasidium* sp. | 1 | - | - | 1 |
| *Cephalosporium* sp. | 1 | - | - | 1 |
| *Drechslera* sp. | 1 | - | 1 | 2 |
| *Fusarium* sp. | - | - | 4 | 4 |
| *F.oxysporum* | - | - | 1 | 1 |
| *Humicola* sp. | - | - | 2 | 2 |
| *Paecilomyces* sp. | 1 | - | 1 | 2 |
| *P. carneus* | - | - | 1 | 1 |
| *P. lilacinus* | - | 1 | 2 | 3 |
| *Penicillium* sp. | 1 | - | 2 | 3 |
| *P. citrinum* | - | - | 1 | 1 |
| *P. fumiculosm* | - | - | 1 | 1 |
| *P.digitatum* | - | - | 1 | 1 |
| *Pochonia chlamydosporium* | 1 | - | - | 1 |
| *Scopulariopsis* sp. | - | - | 1 | 1 |
| *Scolecobasidium* sp. | - | - | 1 | 1 |
| *Trichoderma* sp. | 7 | - | 3 | 10 |
| *T. harzianum* | - | 3 | 1 | 4 |
| *T. virens* | - | 1 | - | 1 |
| *T. viride* | - | 1 | 1 | 2 |
| *V. lecanii* | - | 1 | - | 1 |
| Unidentified | 17 | - | 5 | 22 |
| **Total** | **57** | **7** | **66** | **130** |

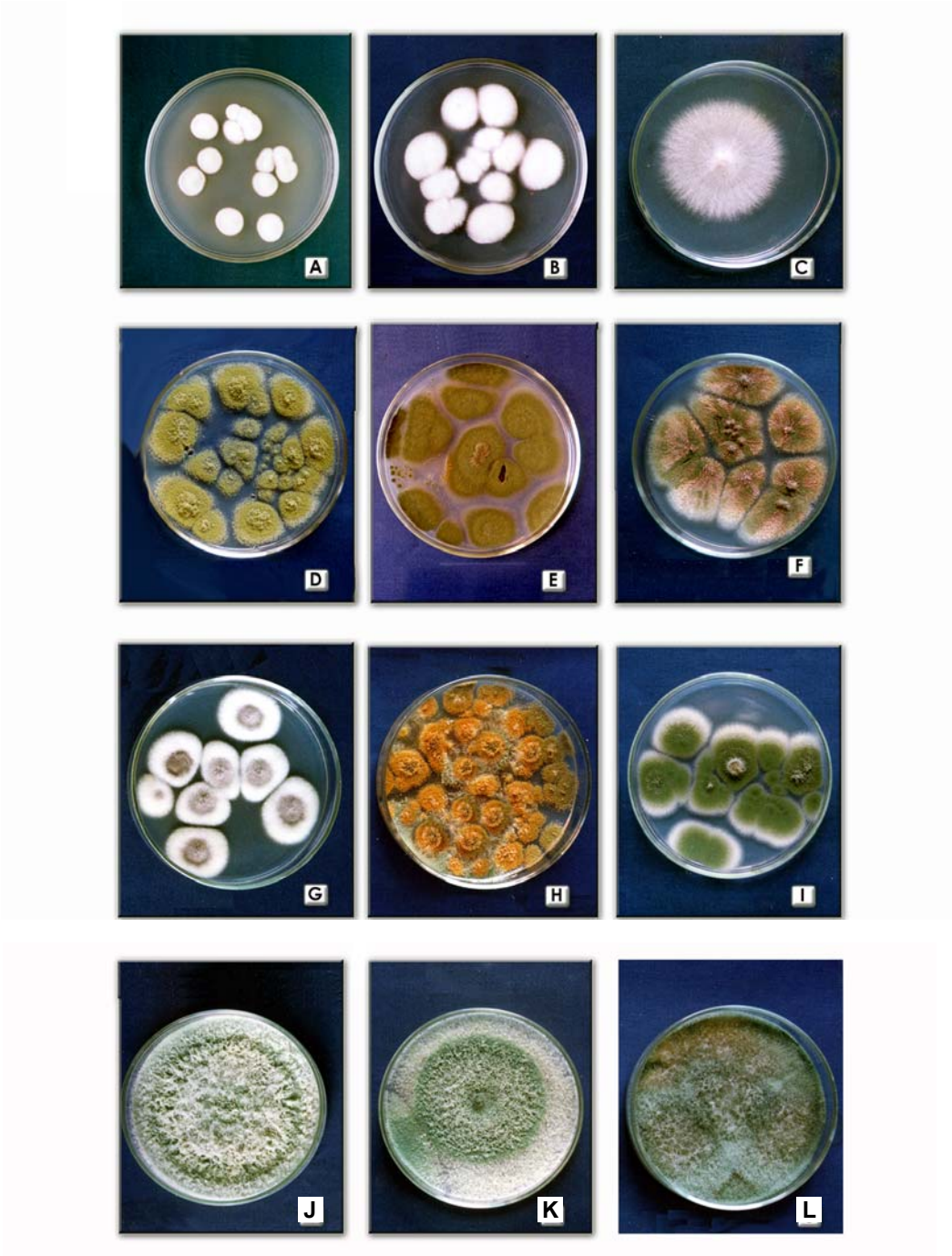



*Fig. 1.* ***Colony morphology of fungal antagonists of plant parasitic nematodes.***

A. *Verticillium lecanii* – Is.35, B. *Pochonia chlamydosporia* – Is.32, C. *Scolecobasidium* sp. – Is.15, D. *Aspergillus* sp. – Is.49, E. *Aspergillus tamarii* – Is.2, F. *Aspergillus fumigatus* – F.6, G. *Aspergillus ustus* – Is.21, H. *Aspergillus fumigatus* – Is.46, I. *Aspergillus restrictus* – Is.7, J. *Trichoderma harzianum* – Is.33, K. *Trichoderma* sp. – Is.16, L. *Trichoderma virens* – Is.39.

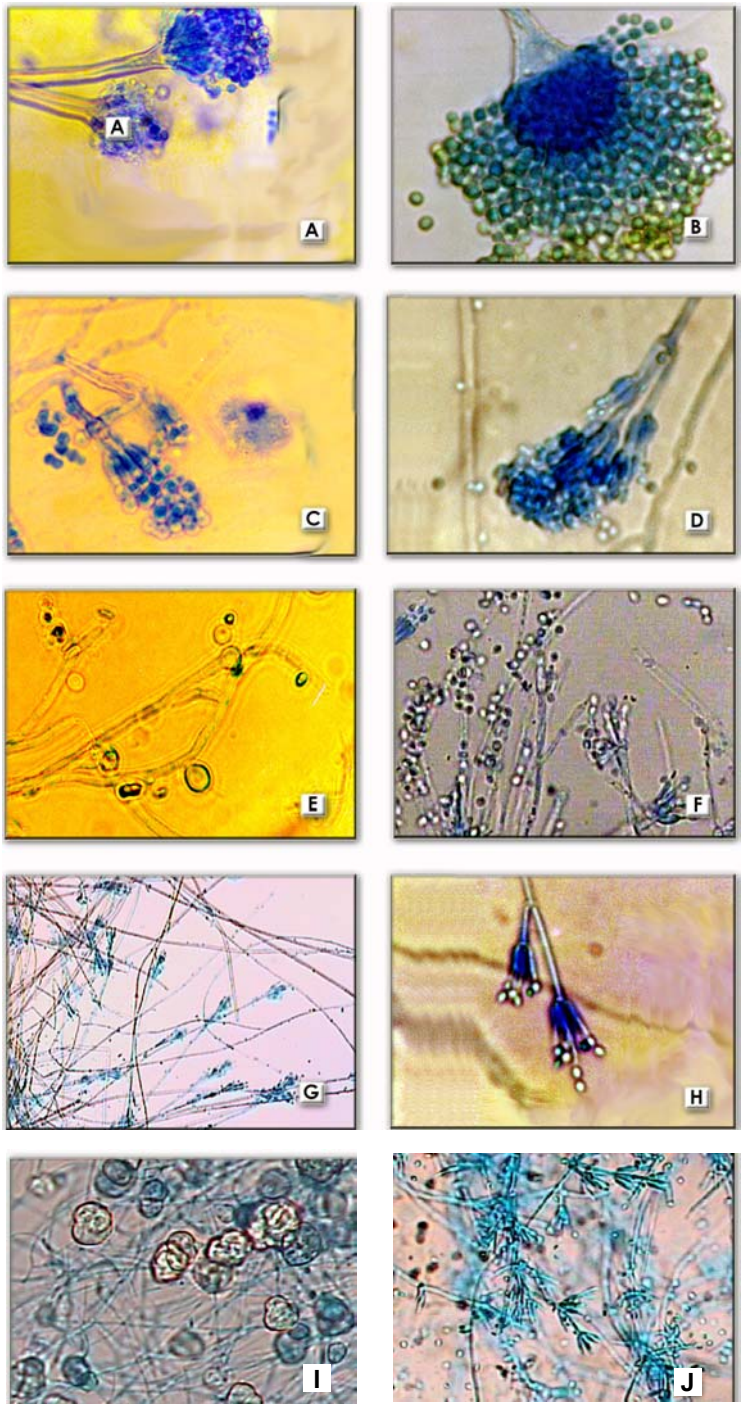



*Fig. 2*. ***Structure of hyphae of fungal antagonists of plant parasitic nematodes.*** A. *Aspergillus ustus* – Is. 21, B. *Aspergillus* sp. – Is.49, C. *Aspergillus tamarii* – Is. 2, D. *Paecilomyces carneus* – Is. 27, E. *Scolecobasidium* sp. – Is. 15, F. *Scopulariopsis* sp. – Is. 14, G. *Paecilomyces lilacinus* – Pl. 1, H. *Paecilomyces lilacinus* – Is.36, I. *Pochonia chlamydosporia* - Is.32, J. *Verticillium lecanii* – Is.35.

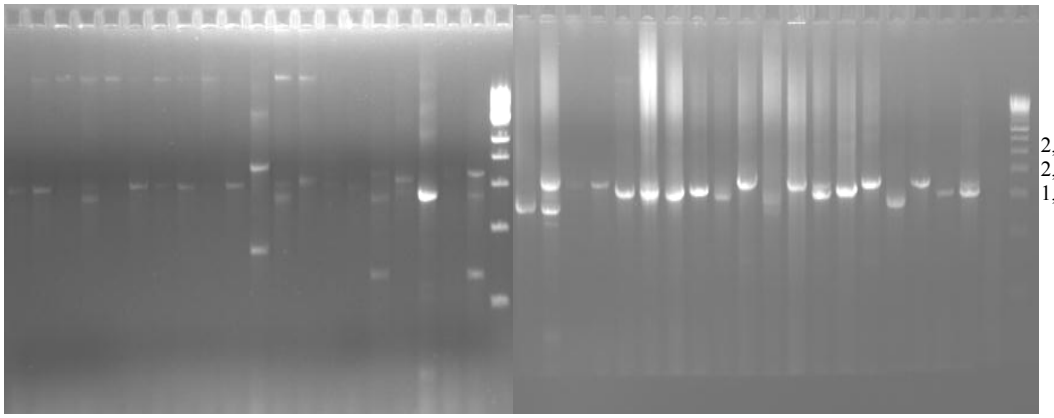
**Variability in *Pochonia***

Mutagenic effects of ethyl methyl sulfonate (EMS) at six concentrations (0, 10, 20, 30, 40, and 50 ppm) were studied on *P. chlamydosporia* and *V. tenerum* isolates. Suppression of colony formation in *V. tenerum* was directly proportional to increasing levels of EMS but concentrations beyond 10 ppm totally inhibited *P. chlamydosporia*. The colonies produced at higher levels of EMS were saved but none of them had any remarkable difference in their growth rate or in any other features.

A laboratory study was conducted to understand the effect of various C and N sources on growth of *Pochonia* isolates. The results of the study showed that the most preferred C and N source were fructose and sodium nitrate, respectively. Copper oxychloride even at 2000 ppm did not inhibit the growth of one of the *P. chlamydosporia* isolates.

**Molecular characterization**

Molecular characterization of 39 isolates of rhizobacteria, diverse in their morphology, phenotypic characters, antibiotic resistance and their antagonistic activity, was completed through ARDRA and rep-PCR (Fig. 3). In the highly discriminatory Rep-PCR, the 39 strains were grouped in to 33 clusters at 70% similarity coefficient (Fig. 4).




| | | | |
|---|---|---|---|
| M- Marker | 11. IISR- 40 | 22. IISR- 528 | 33. IISR- 658 |
| 1. IISR- 51 | 12. IISR- 38 | 23. IISR- 551 | 34. IISR- 685 |
| 2. IISR- 11 | 13. IISR- 3 | 24. IISR- 510 | 35. IISR- 641 |
| 3. IISR- 6 | 14. IISR- 18 | 25. IISR- 292 | 36. IISR- 663 |
| 4. IISR- 8 | 15. IISR- 533 | 26. IISR- 293 | 37. IISR- 683 |
| 5. IISR- 10 | 16. IISR- 549 | 27. IISR- 287 | 38. IISR- 865 |
| 6. IISR- 56 | 17. IISR- 526 | 28. IISR- 289 | 39. IISR- 860 |
| 7. IISR- 35 | 18. IISR- 522 | 29. IISR- 290 | |
| 8. IISR- 15 | 19. IISR- 554 | 30. IISR- 655 | |
| 9. IISR- 13 | 20. IISR- 560 | 31. IISR- 695 | |
| 10. IISR- 44 | 21. IISR- 536 | 32. IISR- 642 | |

Fig. 3. ***Amplification of 16-23 S intergeneic region of the rDNA of 39 isolates of rhizobacteria isolated from the rhizosphere of spices***

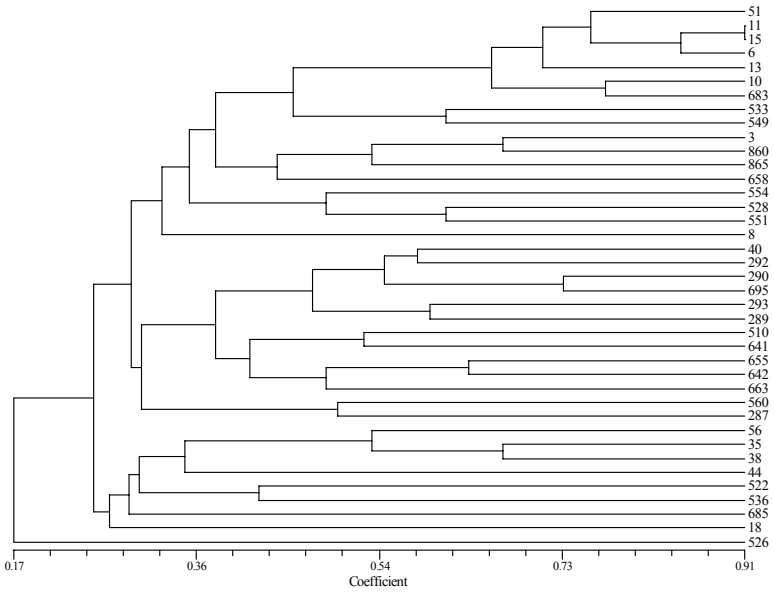



Fig. 4. ***Phylogenetic tree of 39 rhizobacteria based on PCR (REP, BOX and ERIC) studies***

## IN VITRO TESTING

All the newly isolated organisms and several promising isolates obtained from other sources were tested against root knot nematodes under laboratory conditions. For this several new in vitro screening methods were devised. In vitro bioassays to evaluate the efficacy of fungi isolates were performed in different ways like colonizing nematode egg masses with fungi to study their effect on egg hatching, baiting with individual eggs to study the direct parasitization, if any, and toxicity studies using fungal metabolites. For testing nematode parasitization by fungi, egg masses, eggs and juveniles of *M. incognita* were placed separately on agar plates along with the fungus and observed daily for any interaction.

The pathogenicity of fungi to eggs of root-knot nematode, *M. incognita* on water agar varied among species and isolates of various fungi. Sixty-seven out of the 110 isolates screened were not parasitic on females of root-knot nematodes. Significant parasitism on adult females was observed only in three isolates viz. *P. lilacinus* (Pl. 1), *T. harzianum* (Is.33) and *V. lecanii* (Is.35) (Table 2). Majority of them, though colonized the egg masses, did not show high parasitism on nematode eggs. Only four isolates showed remarkable egg parasitism (>25%) and they were two isolates each of *P. chlamydosporia* (Is.32 and Is.34) and *P. lilacinus* (Pl.1 and Is.36) (Fig.5). Another 33 isolates exhibited moderate egg parasitism. Most of these isolates were parasitic on root-knot nematode females too. But none of the screened fungi was parasitic on root-knot nematode juveniles. *P. chlamydosporia* parasitized root knot nematode eggs. Its spores got attached to the nematode eggs and germinated to colonise the inner contents. However, it did not parasitize *M. incognita* juveniles and *R. similis.* Among the *Trichoderma* spp., *T. harzianum, T. longibracheatum, T. koningi, T. viride* and *G. virens* were better colonizers. However no hyphal development was observed on individual eggs or larvae. Distortion of eggs was commonly seen and was more pronounced with regard to *T. harzianum*, *T. viride* and *G .virens* suggesting the involvement of toxic metabolites.

In the bioassay for suppression of hatching, except two isolates (Is. 12 and Is. 27), all the fungi screened showed various degrees of adverse effect on the egg hatch process. However, culture filtrates of all the *Trichoderma* isolates killed the second stages juveniles of *M. incognita* within 24 hours, in a separate study. Other isolates that suppressed root knot nematodes under in vitro conditions were Is.29, Is.30 and Is.61.

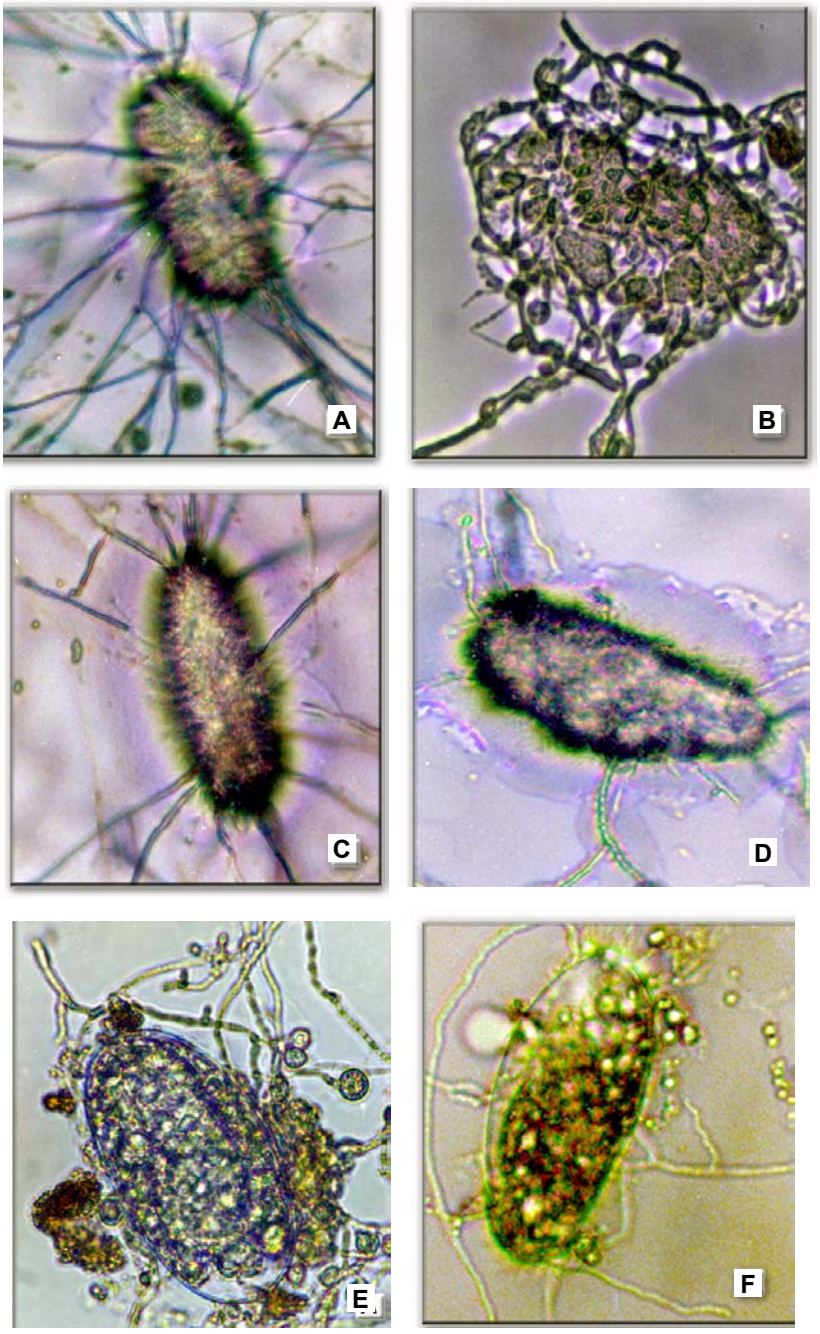



Fig. 5. ***Parasitization of root-knot nematode eggs by egg parasitic fungi.***

A. *Pochonia chlamydosporia* – Is.32, B. *Scopulariopsis* sp. – Is. 14, C. *Paecilomyces lilacinus* – Is.36, D. *Trichoderma harzianum* – Is.33, E. *Fusarium oxysporum* – Is. 11 and F. *Trichoderma virens* – Is.39.

*Table 2.* ***Comparison of nematode suppression mechanisms of promising fungal antagonists.***

| Fungal antagonist | Isolate No. | Parasitism on | | Hatching suppression[b] (%) |
|---|---|---|---|---|
| | | Eggs [a] (%) | Females [a] (%) | |
| *Aspergillus restrictus* | Is.7 | 20.82 | 0.00 | 91.80 |
| *Fusarium oxysporum* | Is.11 | 21.22 | 0.00 | 95.71 |
| *Paecilomyces lilacinus* | Pl.1 | 42.32 | 28.12 | 78.12 |
| | Is.36 | 36.12 | 24.22 | 72.06 |
| *P. chlamydosporia* | Is.32 | 35.63 | 18.36 | 95.45 |
| | Is.34 | 26.42 | 19.48 | 91.24 |
| *Penicillium digitatum* | Is. 23 | 0.00 | 0.00 | 100.00 |
| *Trichoderma* sp. | Is.16 | 14.66 | 10.21 | 97.20 |
| | Is.24 | 16.38 | 0.00 | 93.56 |
| *T. harzianum* | Is.33 | 24.26 | 32.82 | 88.89 |
| *T. viride* | Is.25 | 10.26 | 15.38 | 91.03 |
| *V. lecanii* | Is.35 | 24.36 | 26.48 | 91.89 |
| Unidentified | F.28 | 0.00 | 12.36 | 90.64 |
| | Is.28 | 19.36 | 12.48 | 95.45 |

Values are mean of three replications. Percentage data transformed to arc sine for analysis and converted to original means.

[a]Proportion of eggs / females parasitized by the candidate fungus.

[b]Hatching suppression = (1 - T/C) x 100, where T is the mean of J2 hatching in the treatment and C is the mean of J2 hatching in the control.

Secondary metabolites of *T. harzianum* were extracted using different organic solvents like acetone, chloroform and ethyl acetate from the fungus grown in PDB for 30, 60 and 120 days. The extracts were concentrated, dried and redissolved in the respective solvent and was tested against M. *incognita* and *R. similis* for their toxicity. The ethyl acetate fraction at 50% dilution killed the nematodes within two hours of exposure. Secondary metabolites of five isolates of *Trichoderma* (*T.harzianum*, *Trichoderma* Is. 6, *Trichoderma* mutant Mi 25, *T. harzianum* Is.7 and *T. aureoviride* Tav 34) on screening for their nematicidal activity, varied in their toxicity to second stage juveniles of root knot nematode. The extracts were redissolved in 0.5% dimethyl sulfoxide to overcome the interference of organic solvents. The endogenenous metabolites, extracted by homogenizing mycelial fragments, also showed a similar trend. Different protocols were tried for the assay of hydrolytic enzymes like B-1, 4 endoglucosidase, B-1, 3-glucanase and chitinase of *Trichoderma*.

Fungi like *Trichoderma*, *Fusarium* and *Aspergillus* are not regular candidates for biological control of nematodes. However, many of these saprophytic fungi are reported as occasional parasites of nematodes in literature (Windham *et al*., 1989; Santos *et al*., 1992; Saifullah & Thomas, 1996; Spiegel & Chet, 1998; Pocasangre *et al*., 2000; Ayoub *et al*., 2000; Goswami *et al*., 2001; Zareen *et al*., 2001; Siddiqui *et al*., 2001a; Sharon *et al*., 2001) *Humicola* sp., *Scopulariopsis* sp. and *Scolecobasidium* sp. have been reported as parasites of cyst nematodes (Kuczynska, 1997; Sosknowska & Banaszak, 1998).

In contrast, majority of the fungal isolates (98.7%) inhibited egg hatch at varying levels indicating the involvement of mechanisms other than parasitism. The enzymatic disintegration of vitelline and chitin layers increased the permeability of eggshell and enhanced the mycelial penetration leading to total disintegration of the egg contents. Thus the results indicated that combination of enzymatic and mechanical distortion of eggshell is of great importance in the penetration of the nematode eggshell by the fungi.

Two days old bacterial cultures (multiplied on nutrient broth) were tested for their nematicidal properties, either alive or heat killed, under *in vitro* conditions. Cent percent mortality was observed within 4h of exposure in the case of isolates 6 and five, when live cultures were used. Bacterial isolates 1, 3, 4, 8, 9 and 10 were toxic to root knot nematode juveniles in *in vitro* tests. There was no effect for any of the heat-killed cultures. Twenty and 52 rhizobacteria were screened under in vitro conditions against *R. similis* and *M. incognita*, respectively. Several of them were

highly effective against root-knot nematodes, but not against *R. similis*. Promising isolates of rhizobacteria that suppress both *R. similis* and *M. incognita* were screened against *P. capsici* too. IISR 658, IISR 853 and IISR 865 inhibited *P. capsici* in laboratory tests. They are good P solubilizers too (Table 3).

*Table 3.* ***Promising isolates of rhizobacteria that suppressed Radopholus similis***

| Is. No. | Source | Location | Reaction to | | P solubilization | Production of HCN |
|---|---|---|---|---|---|---|
| | | | Mi | Rs | | |
| 658 | *M.incognita* | Wynad | + | + | + | - |
| 663 | *P. nigrum* | Idukki | + | NT | - | - |
| 853 | *Chromolaena odorata* | Calicut | + | + | + | - |
| 865 | *Strychnos nuxvomica* | Calicut | + | + | + | + |

Culture filtrates of 98 bacterial isolates were tested for their nematicidal activity. Out of these 28 isolates suppressed the egg hatchability by 71.7-92% and increased the nematode mortality by 75-100%. Another 40 isolates had reasonably good (>50% mortality) nematicidal property. Metabolites (volatile and non volatile) of 67 bacterial isolates were also tested for their nematicidal activities. Volatile metabolites play a crucial role in killing the nematodes. Besides, the production of HCN and H2S by these bacteria were also monitored. Out of the 98 isolates screened, only 6 isolates produced HCN. H2S production was observed in another 6 isolates among the 50 tested.

The mode of action and life cycle of *P. penetrans* was studied under in vitro conditions. Root-knot nematode larvae were suspended in a spore suspension of *P. penetrans* for 24-48h. They were then inoculated on 1 month-old black pepper seedlings, planted in steam-sterilised soil. Different stages of the bacterium were observed in the inner contents of the nematodes by sampling the inoculated plants, extracting and crushing the nematodes. Stages like vegetative growth, differentiation, sporulation and maturation were observed.

## GREENHOUSE EVALUATION

A series of greenhouse experiments were conducted using various antagonists of nematodes, found to be promising in the laboratory bioassays, to study their interaction with the test plant and nematodes. For this, the candidate organisms were multiplied on the respective substrate or media and were used to inoculate the test plants. The dosage was determined based on the spore load in unit weight of the substrate.

*Trichoderma* spp.

Four isolates of *Trichoderma* spp. and a *Gliocladium* isolate from cardamom plantations of Kodagu were tested against root knot nematodes of cardamom in a pot trial started during 1993. Among these isolates, isolates C.22 (Is.33) and C.23 were found superior in suppressing root-knot nematode populations than the other isolates. The results are given in Table 4. Isolate C.22 performed better in sterile soil while C.23 was more effective in native (non-sterile) soil. They reduced nematode populations in cardamom roots by 31.50 to 86.80 per cent in sterile soil and by 31.58 to 82.14 per cent in non-sterile soil. In general the nematode multiplication was poor in non-sterile soil, even in control pots. Except C.23, none of the *Trichoderma* isolates was able to improve significantly the growth of cardamom plants in sterile soil (Table 4). However, when plants in sterile soil were infested with root-knot nematodes, all the *Trichoderma* isolates were ineffective in either improving the growth or suppressing the nematodes.

The *T. harzianum* isolates (C.22 and C.23) that were superior in reducing root-knot nematode population in cardamom were effective in *in vitro* bioassays too. Of late, many others also obtained similar results on using *Trichoderma* isolates (Saifullah & Thomas, 1996; Sankaranarayanan *et al*., 1998; Nagesh *et al*., 2001b; Sharon *et al*., 2001). Various mechanisms like antibiosis, competition, mycoparasitism and enzymatic hydrolysis have been suggested for the biocontrol activity of *Trichoderma* spp. against phytopathogenic fungi (Sivan & Chet, 1992). *T. virens* acts against certain pathogenic fungi through the production of antibiotics (Roberts & Lumsden, 1990). The enhanced nematode suppression in native soil suggests the additive role of some microflora present in the soil. Alternately, they might have supported the initial establishment and colonization of the soil by the biocontrol agents. *Trichoderma* isolates, C.20, C.21 and C.23 promoted growth of cardamom seedlings in spite of the nematode infestation. This could be due to the diffusible metabolites released by the fungi. It is reported that some *T. harzianum* isolates have the ability to solubilize insoluble or sparingly soluble minerals by chelation and reduction, which leads to growth promotion (Altomare *et al*., 1999).

Table 4. ***Effect of Trichoderma isolates on growth of cardamom seedlings and root knot nematode development.***

| Treatment | Total Biomass/ Seedling (g) | | | Final nematode level/ g root# | | |
|---|---|---|---|---|---|---|
| | S- | S+ | Difference | S- | S+ | Difference |
| *Trichoderma* Is. 1 | 22.58 | 23.01 | 0.43 | 1.307 | 0 | 1.307* |
| *T. viridae* | 24.62 | 21.08 | 3.54 | 0.451 | 0 | 0.451 |
| *T. harzianum* Is. 1 | 19.04 | 22.12 | 3.08 | 0.512 | 0 | 0.512 |
| *T. harzianum* Is. 2 | 26.75 | 25.00 | 1.75 | 1.304 | 0 | 1.304* |
| Gliocladium sp. | 21.46 | 22.86 | 1.40 | 0.458 | 0 | 0.458 |
| All isolates together | 29.12 | 19.20 | 9.92* | 1.136 | 0 | 1.136* |
| *Trichoderma* Is.1 + N | 17.02 | 15.17 | 1.85 | 2.216 | 2.457 | 0.241 |
| *T. viridae* + N | 18.25 | 17.37 | 0.88 | 2.025 | 2.828 | 0.803 |
| *T. harzianum* Is. 1 + N | 15.21 | 17.92 | 2.71 | 2.074 | 1.943 | 0.131 |
| *T. harzianum* Is. 2 + N | 22.85 | 16.50 | 6.35* | 1.630 | 2.416 | 0.786 |
| *Gliocladium* sp. + N | 15.08 | 18.96 | 3.88 | 2.504 | 2.654 | 0.150 |
| All isolates together + N | 20.54 | 17.08 | 3.46 | 1.748 | 2.572 | 0.820 |
| Nematode alone | 11.33 | 15.33 | 4.00 | 2.380 | 2.818 | 0.438 |
| Check | 20.29 | 19.16 | 1.05 | 1.120 | 0 | 1.120* |
| $LSD_{0.05}$ | 4.50 | 4.50 | - | 0.938 | 0.938 | - |

N- Root knot nematode; S-native soil; S+ sterile soil

* Significant difference between means in a row. # Log transformed values

Among the six isolates of *Trichoderma* screened against nematodes affecting black pepper plants, increased growth response was not observed with any of the isolate (Fig. 6). Slight improvement in growth was noticed only with T.12 isolate. All the growth parameters viz. height of the plant, total biomass and number of nodes plant$^{-1}$ were on par with those of the check plants which received neither the nematodes nor the fungi. Nematode inoculation significantly reduced the growth of black pepper cuttings in all respects. However, T.10, T.12 and to a limited extent T.5 were able to protect the black pepper cuttings against the nematode induced damages. The numbers of colony forming units of *Trichoderma* as well as the total fungi were very high in all treatments excluding the control, the maximum being in P.26 (1.3 x $10^5$ cfu g$^{-1}$ soil) and T.12 (2.81 x $10^5$ cfu g$^{-1}$ soil). But the fungal count came down drastically 12 months after the initial application.

The major reason for the poor performance of *Trichoderma* isolates can be their poor establishment in soil. It is reported that *Trichoderma* application in soil affected the penetration of nematodes and did not inhibit the development of nematodes within the roots (Sharon *et al*., 2001). Large numbers of J2 probably escaped infection because of their high density or the lower concentration of the fungus. So a longer pre-planting incubation period was required to achieve significant nematode control. The nature of the host plant, black pepper, also might have contributed to this. Juvenile penetration into roots of black pepper was reduced only at the beginning of the experiment. The galls due to root-knot nematode infection in black pepper are big in size and therefore the egg masses are embedded in the gall tissues and not accessible to the fungus. Furthermore, frequent high moisture content of the soils in the pots can have a negative effect on sporulation of fungi. The study also revealed the steep decline in *Trichoderma* population towards the end of the experiment. As a result, the subsequent generations of nematodes would have escaped the fungal antagonism, which warrants more frequent application of the biocontrol agents. This study clearly proved that application of *Trichoderma* multiplied on decomposed coffee husk (approximately $10^5$ cfu g$^{-1}$) @ 40g plant$^{-1}$ is too insufficient for black pepper cuttings raised in polythene bags. Repeated applications may be necessary for adequate protection against nematode infestation.

*Pochonia chlamydosporia*

The pot culture trial to study the effect of *Pochonia chlamydosporia* on suppressing root knot nematodes of cardamom was concluded after 10 months. The final results are given in Table 5. Statistically significant differences were not observed in any of the treatments with regard to any of the growth parameters. Nevertheless application

of *P chlamydosporia*, irrespective of its sequence, increased the height of the cardamom plants by 20.44% - 43.08%, the total biomass by 24.20% - 25.40%, and the root weight by 5.24% - 36.82%. The maximum increase in height and root biomass was noticed when *P chlamydosporia* was inoculated first. Pre-inoculation of cardamom plants with *P. chlamydosporia* was found to be more effective in improving the growth of the seedlings. However, a single round of *P. chlamydosporia* application was not sufficient to give extended protection against nematodes.

In another greenhouse trial, *P. chlamydosporia* suppressed *R. similis* in black pepper rooted cuttings. Black pepper cuttings treated with the fungus showed significantly less root damage and very good growth compared to those plants treated with nematodes alone (Table 6).

Inoculation of *P. chlamydosporia* first had relevance as it enabled the fungus to get established and challenge the nematode infection. Even simultaneous inoculation of the fungus and nematodes was able to subside the nematode problem to some extent. Presence of nematodes in roots may stimulate root colonization by *P. chlamydosporia* (Leij *et al*., 1992c). The percentage of egg masses colonized by *P. chlamydosporia* depends on the abundance of egg masses on the root and on the degree of their exposure to the fungus (Bourne *et al*., 1996). Lack of aggressive rhizosphere colonization may be one reason for the inconclusive results obtained here. Therefore, it can be concluded that biocontrol agents should be applied early to young seedlings for ensuring a healthy crop. *P. chlamydosporia* isolates showed marked differences in their ability to colonize roots as well as nematode eggs (Leij & Kerry, 1991). The rhizosphere colonization in non-sterilised soil varied with the fungus strain and with the crop plant (Bourne *et al*., 1994). It is more effective on less susceptible host plants that produce small galls (Bourne *et al*., 1996; Kerry & de Leij, 1992; Viaene & Abawi, 2000). Cardamom, therefore, is an ideal plant that can be protected against root-knot nematode attack by applying fungal biocontrol agents.

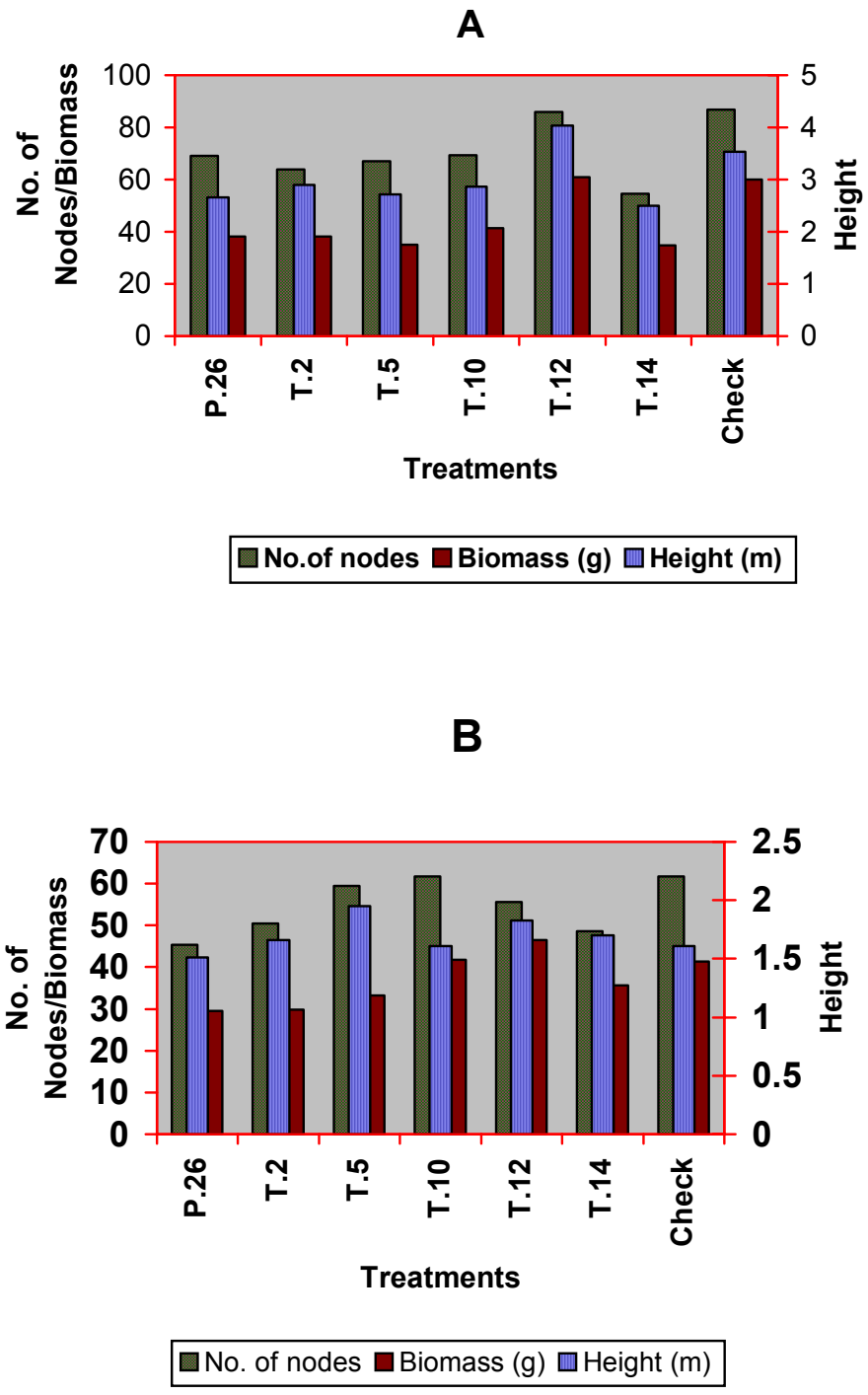



Fig. 6. ***Effect of six isolates of Trichoderma spp. on growth of black pepper rooted cuttings. A. Root-knot nematode inoculated B. Nematode-free plants.***

Table 5. ***Effect of Pochonia chlamydosporia on growth of cardamom seedlings and root knot nematodes (Mean of five replications).***

| Treatment | No. of tillers | Biomass (g) | Root wt. (g) | Nematodes per g root (pf) |
|---|---|---|---|---|
| Check | 3.33 b | 210.00 ab | 31.67 abc | - |
| Vc | 4.60 a | 204.00 ab | 30.00 bc | - |
| Mi | 4.25 ab | 133.33 b | 38.33 ab | 447.75 a |
| Vc + Mi | 3.75 ab | 263.33 a | 33.33 abc | 561.05 a |
| Vc > Mi | 4.00 ab | 261.00 a | 43.33 a | 1207.81 a |
| Mi > Vc | 3.00 b | 210.10 ab | 23.33 c | 500.03 a |

Means in a column followed by he same letter are not significantly different.
Vc – *Verticillium chlamydosporium*; Mi – *Meloidogyne incognita*

Table 6. ***Plant growth and burrowing nematode population in black pepper rooted cuttings treated with Pochonia chlamydosporia and Radopholus similis (mean of 20 replications).***

| Treatment | RLI [a] | Height(cm) | Biomass (g) | | Final nematode population (No.) | |
|---|---|---|---|---|---|---|
| | | | Total | Root | In soil [b] | In roots [c] |
| Rs alone | 4.7 | 30.5 | 11.0 | 0.6 | 8.0 | 110.0 |
| Vc+RS | 1.0* | 38.2* | 19.2* | 1.9* | 3.0 | 450.0* |

[a] Root lesion index (1-5 scale); [b] in 100 cc soil; [c] in one gram root; * Means in the column are significantly different (P=0.05); Rs- *Radopholus similis,* Vc- *Verticillium chlamydosporium*

*Rhizobacteria*

Four isolates of *P. fluorescence*, collected from black pepper soils of Eastern Ghats in Andhra Pradesh were tested against root knot nematodes using tomato as a test plant. Tomato plants were dipped in the bacterial suspension at the time of transplanting. After establishment, these plants were inoculated with 500 second stage juveniles of *M. incognita*. The root weight, number of egg masses and the final nematode level in roots were recorded after two months. Though all the four isolates significantly reduced nematode population level in roots, Isolate No.1 and No.44 were found to possess maximum inhibitory effect on root knot nematodes (Table 7). Excellent root proliferation was observed when plants were inoculated with *P. fluorescens* Is.40.

Another 30 isolates of fluorescent pseudomonads were tested for their interaction with root knot nematodes on black pepper OP seedlings. None of the isolates showed statistically significant improvement in growth of the plant or reduction in nematode multiplication. However, their nature of interaction varied considerably between different isolates tested (Table 8).

The non-significant differences in growth improvement or reduction in root galling, on treating the black pepper seedlings with *P. fluorescens*, can be due to many reasons. First of all the beneficial effects of rhizobacteria might not have been expressed because of the short duration of the experiments (two months). Though not statistically significant, many isolates improved the growth and reduced the nematode damage. The extent and intensity of bacterial colonization determines whether sufficient metabolites will be produced to inhibit, interrupt or stimulate certain processes in the nematodes life cycle (Becker *et al*., 1988; Weller, 1988; Defago & Keel, 1993). The late introduction of bacteria in all these experiments (except the last one) might have affected the colonization level and thereby the synthesis of metabolites.

Similarly, inoculum density, light quality, watering, soil microbial community, plant nutritional status, soil pH, temperature and survival of applied bacteria are also reported to influence the antagonistic activity of these rhizobacteria (Weller, 1988; Oostendorp & Sikora, 1989; Deacon, 1991). Intergeneric and interspecific competition between rhizobacteria may influence bacterial colonization on the root surface (Racke & Sikora, 1992). Similar results were observed against root-knot nematodes (Zavaleta-Mejia & van Gundy, 1982; Becker *et al*., 1988; Santhi & Sivakumar, 1995; Santhi *et al*., 1999); citrus nematode (Santhi *et al*., 1998); cyst nematodes (Gokte & Swarup, 1988; Oostendorp & Sikora, 1989) and rice root

nematode (Ramakrishnan & Sivakumar, 1998). It is also reported that nematodes like *Meloidogyne*, which are with multiple generations and cause damage throughout the season, are more difficult targets for *P. fluorescens* (Sikora, 1992). Under greenhouse conditions, soil drenches with the aqueous cell suspension or cell-free culture of *P. aeruginosa* resulted in a considerable reduction in nematode population densities in soil and subsequent root-knot development due to *M. javanica* (Siddiqui & Haq, 2001).

Five isolates of rhizobacteria (IISR 522, IISR 528, IISR 658, IISR 853 and IISR 859) having dual nematicidal action (suppressing both *R. similis* and *M. incognita*) were short-listed from a collection of 291 isolates (Table 9). Chitin based formulations of five promising rhizobacteria were tested against *R. similis* and *M. incognita* in another greenhouse trial. LS255 and LS260 caused maximum suppression of root-knot nematodes but LS255 and 256 were the best in controlling *R. similis*.

*Pasteuria penetrans*

Effect of *Pasteuria penetrans* on the root knot nematode population was tested in a pot culture trial. The results showed that the bacterial inoculation simultaneously with root knot nematodes and two weeks after nematode inoculation significantly reduced the nematode populations. However, significant growth enhancements were noticed in treatments where *P. penetrans* was inoculated after nematodes (Table 10). The results also clearly indicated that plants inoculated with increasing levels of *P. penetrans* correspondingly decreased the root galling and root-knot nematode population in cardamom plants. However, the juvenile populations in soil varied widely. The endospore attachment on $J_2$ at the end of the experiment was generally low and ranged from 0.57 to 3.1 endospores $J_2^{-1}$. Various stages in the life cycle of the bacterium could be identified on examining the body contents of infected nematodes. The data also showed that infestation by root-knot nematodes, if ignored, could appreciably decrease the growth and biomass production of cardamom seedlings.

The role of *P. penetrans* in suppressing plant parasitic nematodes has been tested in many crops (Chen & Dickson, 1998). The reduction in nematode levels was inversely proportional to the inoculum levels of *P. penetrans* in crops like peanut (Chen, Z.X. *et al*., 1997). However, the high incidence of nematode juveniles in soil with low spore encumbrance was quite surprising. Single application of *P. penetrans* was not sufficient to sustain the bacterial inoculum in soil. Watering is reported to affect the spore distribution in soil (Davies *et al*., 1991).

*Table 7.* ***Evaluation of four isolates of fluorescent pseudomonads for control of root-knot nematodes infesting tomato.***

| Bacterial isolate | Root wt (g) | | | No. of egg masses | Nematodes $g^{-1}$ root (Pf) |
|---|---|---|---|---|---|
| | N- | N+ | Mean | | |
| Is. 1 | 2.55 bc | 2.61 abc | 2.58 bc | 3.33 b | 1005.93 c |
| Is. 22 | 2.23 c | 1.82 c | 2.02 c | 0.50 bc | 2268.86 b |
| Is. 40 | 2.62 abc | 3.69 a | 3.15 ab | 28.71 a | 2534.13 b |
| Is. 44 | 1.98 c | 2.78 abc | 2.38 bc | 0.0 c | 2375.84 b |
| Control | 3.77 a | 3.19 ab | 3.48 a | 30.33 a | 5558.04 a |

Data are means of three replications. Means followed by the same alphabets are not significantly different. N- without nematodes, N+ with nematodes.

Table 8. ***Evaluation of different isolates of fluorescent pseudomonads***

| Treatment | Nature of interaction & isolate No. |
|---|---|
| Fluorescent pseudomonads alone | Improved growth of the plant – Is. No. 27, 29 & 34 |
| Fluorescent pseudomonads + Root knot nematodes | No effect on nematodes but improved growth of the plant – Is. No. 2, 13, 30, 33, 36 & 43.<br>Suppressed nematodes but no effect on plant growth – Is. No. 12, 23 & 51.<br>Suppressed nematodes and improved plant growth – Is. No. 7, 10, 14, 20, 26-28, 31, 358~49 |

Table 9. ***Promising isolates of rhizobacteria short listed for field evaluation***

| Rhizobacteria | Nematode population / g root | |
|---|---|---|
| | *R. similis* | *M. incognita* |
| IISR 552 | 86.57 | 14.80 |
| IISR 528 | 17.08 | 12.00 |
| IISR 658 | 6.52 | 0.00 |
| IISR 853 | 5.23 | 0.00 |
| IISR 859 | 4.16 | 0.00 |
| Nematode alone | 1008.20 | 124.80 |
| Absolute control | 0.00 | 0.00 |

Table 10. ***Effect of Pasteuria penetrans on growth of cardamom (Mean of five replications)***

| Treatment | No.of tillers | Height (cm) | Root wt (g) | Total biomass(g) |
|---|---|---|---|---|
| Check | 1.96 a | 75.17 bc | 12.30 a | 41.02 b |
| MI alone | 1.50 a | 51.40 d | 8.70 a | 29.42 c |
| Pp alone | 2.25 a | 81.17 b | 10.88 a | 40.22 b |
| Pp>MI(2W) | 2.50 a | 69.00 bc | 8.49 a | 24.70 c |
| Pp>MI(4W) | 2.00 a | 92.40 a | 9.53 a | 39.47 b |
| Pp+MI | 2.29 a | 64.00 c | 10.64 a | 16.25 d |
| MI>Pp(2W) | 2.14 a | 73.00 bc | 14.03 a | 54.52 a |
| MI>Pp(4W) | 2.00 a | 76.00 bc | 12.49 a | 59.10 a |

MI – *Meloidogyne incognita* Pp – *Pasteuria penetrans*; Means followed by the same letter in a column are not significantly different.

**FIELD EVALUATION**

A new trial has been laid out in a root knot nematode infested black pepper garden in Pulpally, Wyanad where the mean nematode population was 1615.97 nematodes per gram of root and 13.11 $J_2$ per 100 cc soil. The mean incidence of foliar yellowing in the plot at the start of the experiment was 72.92%. Three biocontrol agents viz. *T.harzianum, P. chlamydosporia* and *P. penetrans* were evaluated in comparison with phorate. Yield and health status of vines were generally superior in plots treated with biocontrol agents (Fig.7). The highest yield was obtained in *P. chlamydosporia* treated plots, which was significantly higher than that of control plots (Table 11). The highest mean yield (5.14 kg $vine^{-1}$) was obtained in *P. chlamydosporia* treated plots followed by combined application of phorate and potassium phosphonate (4.20 kg $vine^{-1}$), which were significantly higher than that of control plots. The root-knot nematode population decreased in all treatments compared to the initial population but the reduction was not statistically significant in any of the treatment. However, the lowest mean population of root-knot nematodes in black pepper roots was observed in phorate + potassium phosphonate treated plants followed by *P. penetrans* treated vines. After 4 years of field evaluation, the nematode level was the lowest in *P. chlamydosporia* treated plots. The mean incidence of yellowing in plots treated with biocontrol agents varied from 17.3-37.3% while that in plots treated with pesticides and untreated control ranged between 32.8-50.5%. The maximum improvement in the crop stand (75.2% healthy vines) was observed in plots treated with *P. chlamydosporia* followed by *T. harzianum* (75.6% healthy vines). The economics of biological control of nematodes of black pepper has been worked out. Application of *P. chlamydosporia* has a cost benefit ratio of 7.12 in comparison with 3.72 of phorate application and 2.85 of *Trichoderma* application.

*P. chlamydosporia* was highly effective compared to *T. harzianum* in suppressing nematodes and increasing the yield of black pepper. *T. harzianum* is reported to have a limited capacity to grow in the rhizosphere (Chao *et al*., 1986; Ahmad & Baker, 1987). Moreover, it is suggested that the main anti-nematode activity caused by *T. harzianum* takes place in soil and not within roots (Sharon *et al*., 2001). In a crop like black pepper which is highly susceptible to root-knot nematodes and produce very large compound galls, the soil phase of these nematodes is for a very brief spell. Many of the nematode antagonistic fungi are reported to be good phosphate solubilizers and their application not only helps in controlling nematodes but also promotes plant growth by increasing the P availability in soil (Somasekhar *et al*., 1998). *P. penetrans,* since deployed as a single control measure, failed to give consistent and durable control of nematodes in perennials. It has been evaluated in

perennial crops like kiwi (Stirling, 1984; Verdejo, 1992). Under a continuous crop it may require more time to suppress the nematodes (Oostendorp *et al*., 1991). Besides, temperature and pH can also influence the endospore attachment on nematodes (Hatz & Dickson, 1992; Serracin *et al*., 1997; Orui, 1997; Chen & Dickson, 1998).

A trial was conducted in a cardamom nursery at Igoor, Kodagu, to evaluate the performance of *P. chlamydosporia* and *P. penetrans* under field conditions. The results clearly demonstrated the vast potential of both these organisms as effective biocontrol agents of root knot nematodes. Significant reduction in nematode population was observed in plots where *P. chlamydosporia* was applied. Although there was no significant improvement in the growth of cardamom seedlings with either of the biocontrol agents, the maximum growth improvement was obtained with *P. chlamydosporia* treatment. *P. chlamydosporia* could be successfully reisolated from the soil 6 and 12 months after application, which again proved their rhizosphere competence in the soil (Table 12).

The low population levels of root-knot nematodes at the initial phase of the experiment might have affected the establishment of *P. chlamydosporia.* This fungus is more abundant on roots infected by nematodes compared with those that are healthy (Kerry, 2001). The host status of the plant species also plays a key role in the establishment of the fungus (Bourne *et al*., 1996; Bourne & Kerry, 1999). On the other hand, *P. penetrans* failed to induce any change in the nematode population or in the growth of cardamom seedlings indicating the non-suitability of this biocontrol agent in situations where the initial nematode level is very high and rapid kills are needed. Amplification of *P. penetrans* to suppressive levels requires longer duration. Oostendorp *et al*. (1991) reported three years as the minimum period for the bacterium to yield adequate control. *P. penetrans* is a mesophilic bacterium with an optimum temperature between 28ºC and 35ºC (Hatz & Dickson, 1992; Serracin *et al*., 1997). Relatively high temperatures generally favour endospore attachment, germination and pathogenesis (Chen & Dickson, 1998). Therefore, temperature below 25ºC may not be ideal for their development and multiplication. Initial nematode density also plays a crucial role in the establishment and multiplication of *P. penetrans* (Gowen *et al*., 1998). This may be one of the reasons for the poor performance of this obligate nematode parasite at Kodagu.


Fig.7. ***Field Evaluation of Trichoderma harzianum, Verticillium chlamydosporium and Pasteuria penetrans in a Root-Knot Nematode Infested Black Pepper Garden in Pulpally, Wyanad, Kerala***.

A. Black pepper vines treated with *Trichoderma harzianum* (Is.33), B. Vines treated with *Pochonia chlamydosporia* (Is.32), C. *Pasteuria penetrans* (Pp. 1) applied black pepper, D. Plot where potassium phosphonate and phorate were applied, E. Phorate treated black pepper vines and F. Control plot (no pesticides and no biocontrol agents)

*Table 11.* ***Crop stand and yield of black pepper in a biocontrol field trial at Pulpally, Kerala (Mean of three replications)***

| Treatment | Vines showing yellowing (%) | | | | | Healthy vines (%) | | | | | Yield (kg-green) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1998 | 1999 | 2000 | 2001 | Mean | 1998 | 1999 | 2000 | 2001 | Mean | 2000 | 2001 | Mean |
| *Trichoderma harzianum* | 46.67 (43.09) | 31.69 (34.26) | 17.30 (24.60) | 36.93 (36.91) | 32.44 (34.72) | 53.33 (40.91) | 68.31 (55.74) | 72.54 (58.42) | 63.06 (53.08) | 64.68 (53.53) | 3.46 | 3.94 | 3.70 |
| *Pochonia chlamydosporia* | 58.18 (49.71) | 33.69 (35.48) | 24.82 (29.88) | 20.50 (26.92) | 33.72 (35.50) | 41.82 (40.29) | 55.32 (48.06) | 40.18 (39.34) | 79.47 (63.08) | 54.69 (47.69) | 4.66 | 5.62 | 5.14 |
| *Pasteuria penetrans* | 59.41 (50.43) | 47.98 (43.84) | 37.27 (37.63) | 22.82 (28.34) | 41.42 (40.06) | 40.18 (39.34) | 50.63 (45.36) | 59.49 (50.47) | 77.18 (61.66) | 57.32 (49.21) | 3.43 | 3.18 | 3.30 |
| Phorate + Potassium phosphonate | 83.81 (66.27) | 66.29 (54.51) | 50.51 (45.29) | 34.44 (35.90) | 59.53 (50.49) | 13.27 (21.36) | 31.09 (33.89) | 44.87 (42.05) | 65.56 (54.10) | 37.65 (37.85) | 3.04 | 5.36 | 4.20 |
| Phorate | 86.40 (68.36) | 67.43 (55.20) | 32.86 (34.98) | 15.25 (21.60) | 50.06 (45.03) | 10.92 (19.30) | 32.57 (34.80) | 67.42 (55.01) | 84.75 (68.40) | 48.91 (44.38) | 2.81 | 4.76 | 3.79 |
| Control | 92.41 (74.01) | 66.95 (54.91) | 41.04 (39.84) | 32.39 (34.67) | 60.15 (50.86) | 7.59 (15.99) | 32.56 (34.79) | 54.88 (47.80) | 67.61 (55.38) | 38.74 (38.49) | 2.09 | 3.63 | 2.86 |
| Mean | 72.92 (58.64) | 52.38 (46.37) | 33.51 (35.37) | 26.10 (30.72) | - | 25.81 (30.53) | 44.96 (42.11) | 56.70 (48.85) | 73.91 (59.28) | - | 3.25 | 4.42 | - |
| LSD0.05 | Years (Y)– 8.22; Treatments (T) – 9.93<br>Y x T – N.S. | | | | | Y – 9.19; T – 9.06<br>Y x T – N.S. | | | | | Y – 1.0 2; T – 1.16<br>Y x T – N.S. | | |

Figures in parentheses are arc sine transformed values.

Another field trial was laid out at Peruvannamuzhi using four fungal isolates viz. *P. chlamydosporia*, *T. harzianum*, *P. lilacinus* and *Scopulariopsis* sp. *P. chlamydosporia* and *Scopulariopsis* sp. were promising in reducing the foliar yellowing and reducing the nematode infestation in black pepper.

Promising isolates of *P. chlamydosporia*, *T. harzianum*, *Fusarium oxysporum* and *P. lilacinus* were evaluated in two ginger fields too. Though none of them could significantly increase the yield of ginger, the highest yield was obtained with the application of *P. chlamydosporia* (Table 13).

An observation trial was laid out in the Experimental Farm, Peruvannamuzhi to study the management of nematode pests of black pepper by deploying five promising isolates of rhizobacteria (IISR 522, IISR 528, IISR 658, IISR 853 and IISR 865). Among these, IISR 522, IISR 528 and IISR 658 were superior in suppressing plant parasitic nematodes and significantly reducing the foliar yellowing in black pepper vines.

Two more new trails were laid out at Peruvannamuzhi to evaluate the performance of ginger (variety 'Himachal') and turmeric (variety 'Prathibha') when they are supplemented with promising PGPR strains. Ten PGPR strains were evaluated for their efficacy to improve the plant growth and suppress soil borne diseases and nematodes. There were four replications with a plot size of two beds of 3m x 1 m. The rhizobacteria were multiplied on molasses and applied at the time of sowing (@ 2.5 l / bed) and after two months. Observations were recorded on the germination of seeds and yield. Incidence of pests and diseases was also monitored. The germination of ginger seeds was significantly improved consequent to the application of rhizobacteria (Table 14). The maximum germination was observed with IISR 853 in both ginger and turmeric. Application of IISR 6, IISR 13, IISR 51, IISR 149, IISR 853 and IISR 866 increased the ginger yield by 33.9 – 62.7%. However, in turmeric, significant improvement in either germination or yield could not be obtained with any of the bacterial strains.

Further 10 promising isolates of rhizobacteria (IISR 6, IISR 13, IISR 51, IISR 522, IISR 658, IISR 853, IISR 859 & IISR 866; LS 149 & LS 151) were evaluated in a field trial using ginger (Himachal) and turmeric (Prathibha). The bacterial suspension (~$x10^{11}$ cfu/ml) multiplied on nutrient broth, was drenched @ 2.5 l / bed twice, immediately after sowing and after 2 months. Unlike in the previous year, only two isolates (IISR 522 and 658) significantly improved the yield of ginger. However in turmeric IISR 853 followed by LS.151 and IISR 6 gave the maximum yield (Table 15). Nematode distribution was not uniform in these plots.

Table 12. ***Effect of Pochonia chlamydosporia and Pasteuria penetrans on growth of cardamom seedlings and incidence of root knot nematodes (mean of four replications).***

| Treatment | No. of tillers | Height(cm) | Nematodes/ g root (pf) |
|---|---|---|---|
| Check1 (Uninoculated) | 2.65 a | 67.60 ab | - |
| Check2 (Inoculated) | 2.40 a | 55.90 bc | 323.33 a |
| Vc alone | 3.15 a | 64.80 ab | - |
| Vc + Mi | 2.60 a | 60.65 abc | 47.75 b |
| Pp alone | 2.35 a | 50.12 c | 116.75 ab |
| Pp + Mi | 2.80 a | 70.50 a | 16.67 b |

Means in a column followed by the same letter are not significantly different.
Vc – *Verticillium chlamydosporium*; Mi – *Meloidogyne incognita*

Table 13. ***Evaluation of fungal bioagents in a ginger field trial at Peruvannamuzhi.***

| Treatment | Height (cm) | Tillers | Yield (kg – 3x1 m bed) | Nematodes / g root |
|---|---|---|---|---|
| Control | 74.98 a | 9.39 a | 5.15 bc | 24.40 b |
| *P. chlamydosporia* | 73.99 a | 9.32 a | 5.83 c | 1.95 a |
| *T. harzianum* | 74.75 a | 8.75 a | 3.98 a | 6.97 ab |
| *F. oxysporum* | 73.49 a | 9.36 a | 4.75 ab | 23.67 b |

Table 14. ***Evaluation of promising rhizobacteria for growth and yield of ginger and turmeric during 2003-04.***

| Isolate No. | Ginger | | *Turmeric* | |
|---|---|---|---|---|
| | Germination (%) | Yield (kg/3m$^2$) | Germination (%) | Yield (kg/3m$^2$) |
| IISR 6 | 76.12a | 4.38 b | 92.16ab | 6.41 ab |
| IISR 13 | 78.98a | 5.32 a | 95.10 a | 6.23 ab |
| IISR 51 | 77.76a | 4.90 ab | 85.48 b | 6.00 ab |
| IISR 149 | 82.54a | 4.27 bc | 93.79ab | 5.92 ab |
| IISR 151 | 73.97a | 4.27 bc | 88.12ab | 5.59 ab |
| IISR 853 | 84.10a | 4.38 b | 93.77ab | 5.81 ab |
| IISR 859 | 73.57a | 4.29 bc | 87.14ab | 6.18 ab |
| IISR 866 | 77.04a | 4.48 ab | 92.62ab | 5.82 ab |
| IISR 522 | 81.15a | 4.12 bcd | 91.96ab | 4.51 b |
| IISR 658 | 77.36a | 3.30 d | 92.40ab | 5.97 ab |
| Control | 59.32b | 3.40 cd | 93.88ab | 6.47 a |

Table 15. ***Field evaluation of rhizobacteria in ginger and turmeric fields during 2004-05.***

| Treatment | Ginger | | | | Turmeric | | | |
|---|---|---|---|---|---|---|---|---|
| | Germ. (%) | Height (cm) | Tillers | Yield* (kg/3m$^2$) | Germ. (%) | Height (cm) | Tillers | Yield (kg/3m$^2$) |
| IISR 6 | 79.0bc | 64.6a | 4.7a | 2.73a | 99.0ab | 1.55ab | 2.4b | 17.45ab |
| IISR 13 | 83.5c | 67.1abc | 4.8ab | 2.97ab | 100.0b | 1.55ab | 2.1a | 16.83ab |
| IISR 51 | 72.7b | 65.5ab | 5.3abc | 2.62a | 100.0b | 1.70ab | 2.1a | 15.26a |
| Kl. 149 | 82.0bc | 73.0bc | 5.6bc | 3.27abc | 100.0b | 1.68ab | 2.2ab | 16.70ab |
| Kl. 151 | 73.6b | 70.9abc | 5.8c | 3.46abc | 99.5ab | 1.66ab | 2.1a | 17.66ab |
| IISR 853 | 72.9b | 73.0bc | 5.6bc | 3.60abc | 98.1a | 1.80b | 2.3ab | 20.25b |
| IISR 859 | 72.5b | 72.2abc | 5.3abc | 3.34abc | 99.5ab | 1.47ab | 2.1a | 16.30ab |
| IISR 866 | 61.7a | 70.3abc | 5.6bc | 2.84a | 99.5ab | 1.44ab | 2.2ab | 15.72ab |
| IISR 522 | 76.9bc | 72.5abc | 5.5bc | 4.00bc | 99.5ab | 1.38a | 2.3ab | 16.66ab |
| IISR 658 | 74.6bc | 74.0c | 5.8abc | 3.68abc | 100.0b | 1.50ab | 2.2ab | 15.72ab |
| Control | 83.3c | 73.7c | 5.4c | 4.22c | 99.4ab | 1.55ab | 2.3ab | 16.00ab |

## MASS MULTIPLICATION

Studies on mass-multiplication of *P. chlamydosporia* on solid substrates like rice bran, tapioca powder, decomposed coir compost and neem oil cake have shown that rice bran as the best substrate (no. of chlamydospores – 2.24 x $10^7$) (Fig. 8). But in tapioca powder, there were no chlamydospores. The highest production of conidiospores and chlamydospores was observed in rice bran (1.05 x $10^9$ and 2.24 x $10^7$, respectively). Both neem oil cake and coir compost also supported good multiplication of *V. chlamydosporium*. The variability in multiplication of the fungus in different substrates is due to the nutrient composition of these substrates. As expected, rice bran contained maximum N and P followed by neem oil cake. Among the four substrates, tapioca powder had the lowest N content and this can be the reason for the poor chlamydospores production in it.

Synergistic effect of some plant extracts having nematicidal effect on mass multiplication of *P. chlamydosporia* was studied. Aqueous extracts of *Azadirachta indica* and *Chromolaena odorata* supported good growth and sporulation of *P. chlamydosporia.* The mycelial production was the highest in PDB followed by that in *Lantana camera* extract. On the contrary, sporulation was very high in all the plant extracts compared to PDB (Fig.9). The highest chlamydospores production (3.98 x $10^5$) was observed in *C. odorata* (stock) followed by 50% of *N. alata* (1.20 x $10^5$). In a similar study, Sosamma and Jayasree (1999) reported that plant leaves like *Hyptis suaveolens*, *C. odorata* and *Mimosa invisa* supported maximum sporulation of *Paecilomyces lilacinus*, another nematophagous fungus. As observed in the present study they obtained the minimum spore production in *G. maculata*, indicating the presence of some antifungal compounds in that plant.

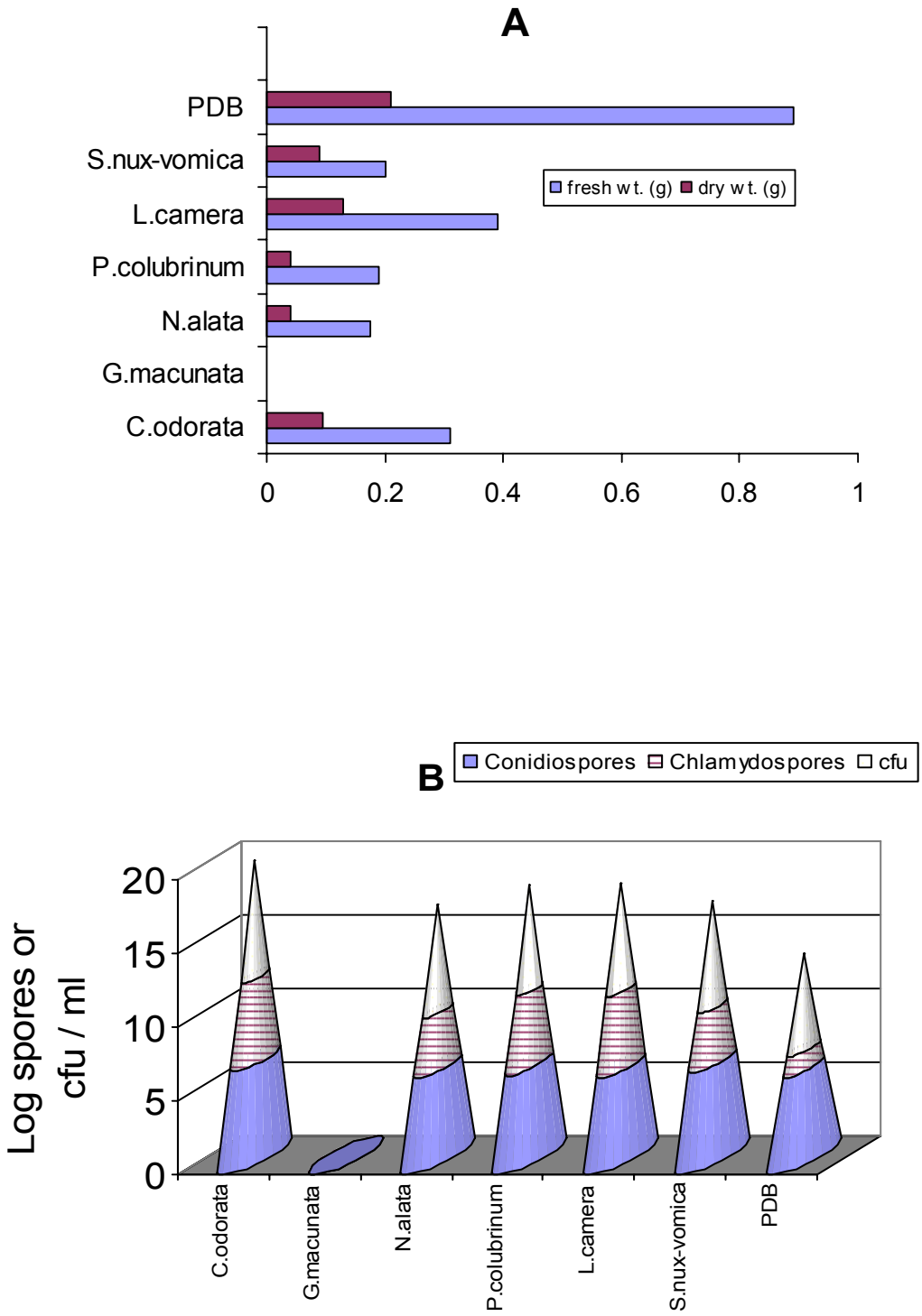



*Fig. 8 .* ***Growth and multiplication production of Pochonia chlamydosporia in aqueous plant extracts.***
A – Production of mycelia and B –Production of spores

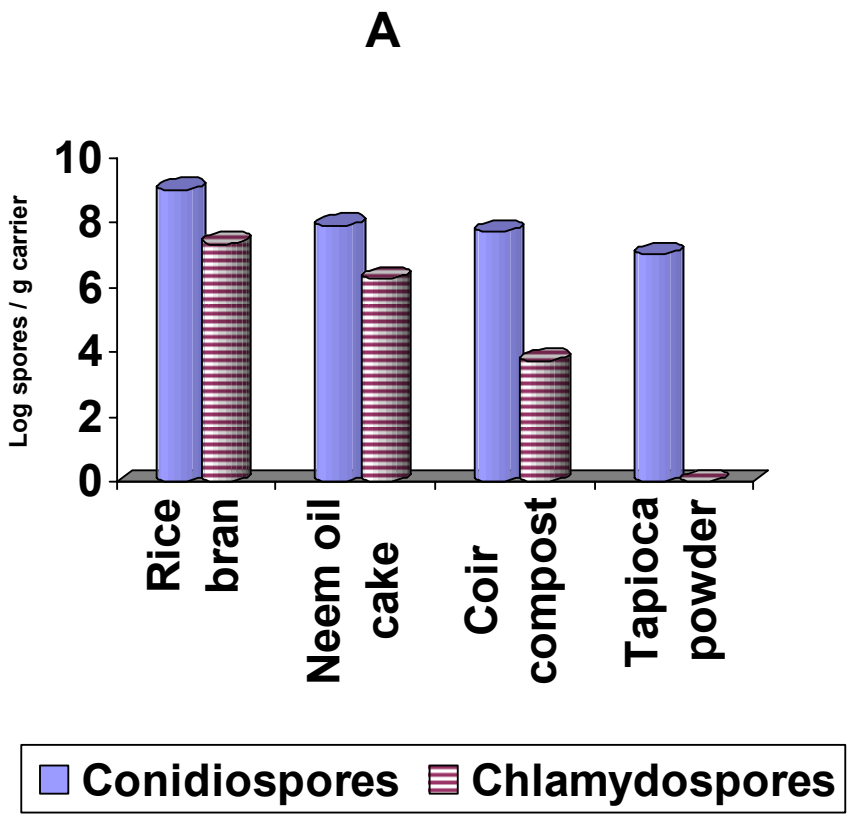



Fig. 9. Mass multiplication of *Verticillium chlamydosporium* on four solid substrates.

# ANNEXURE II

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# ANNEXURE II

## RESEARCH PUBLICATIONS