RPF - III
(PERFORMA FOR SUBMISSION OF FINAL REPORT OF RESEARCH PROJECTS)

Part - I: General Information

800 Project Code

8001 Institute Project Code No. : Nema. V (813)
8002 ICAR Project Code No. :

801 Name of the Institute and Division

8011 Name and address of Institute : Indian Institute of Spices Research, Kozhikode
8012 Name of Division/Section : Crop Protection/Nematology
8013 Location of the Project : Kozhikode

802 Project Title **:** Survey and identification of efficient entomopathogenic nematodes (EPNs) against insect pests infesting ginger and turmeric

803 Priority Area : Pest management

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 **:** 4 years

8051 Date of start of project : 22nd October 2008
8052 Likely date of completion of project : 31st March' 2012
8053 Period for which report submitted : 2008 -12

806 Total cost /Expenditure Incurred (Give reasons for variation, if any from original estimated cost): 16.96 Lakh

Expenditure to date : Rs. 22.35 Lakh*

* The variation in expenditure incurred was due to the budget estimate which was based an old pay scale of scientists and technical staff.

807: Executive Summary

Ginger and turmeric suffer from ravages of a variety of insect pests. At present most of the available control measures are predominantly insecticidal. With the passing of time and realizing the harmful effects of insecticides, biocontrol agents are being deployed with varying degree of success. Entomopathogenic nematodes (EPNs) have got little attention by researchers though they have a great potential in reducing pest population and with little manipulation their role can be enhanced. Use of EPNs will be an ideal alternative, which will be economical and result in long term pest control system without risk to non target organisms associated with crops.

The objective of the project that was in progress during 2008-12 was survey and collection of soil samples for detection of EPNs, identification of EPNs, infectivity of EPNs against insect pests infesting ginger and turmeric and mass production of EPNs on artificial media. The salient achievements under the project are following.

Isolation of EPNs: One hundred and fifty seven soil samples were collected from ginger and turmeric rhizosphere from different locations of Kozhikode, Wyanad, Kottayam, Idukki (Kerala), Kodagu (Karnataka), Coimbatore (Tamil Nadu), Guwahati (Assam), Faizabad (Uttar Pradesh) and Barapani (Meghalaya) districts. Out of 157 soil samples baited out, only eight samples were found to be positive to entomopathogenic nematodes. Among these strains of EPN, three EPNs were from Kozhikode district, three from Idduki district and one each from Waynand and Faizabad district.

Identification of EPNs: Out of eight isolated species of EPN, two species belong to genus *Steinernema*; one to *Heterorhabditis* and five to *Oscheius*. Among them, two new species *viz.*, *Steinernema ramani* sp. n. and *Oscheius gingeri* sp. n. have been identified on the basis of morphometric and molecular characterization.

Infectivity of EPNs: Among the eight EPN isolates, only four isolates viz., *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 07 and 08) were promising against shoot borer larva, whereas *Oscheius* sp. (IISR 07) and *Steinernema* sp. (IISR 02) were promising against shoot borer pupa.

Mass production of EPNs: Multiplication of infective juveniles of eight isolates of EPN in different artificial media *viz.*, Wouts medium, egg yolk medium, dog biscuit medium, agar agar medium and wheat flour medium were tested. Among these media, egg yolk was the best media for the multiplication of IJs followed by Wouts media.

808 Key words: Entomopathogenic nematodes, shoot borer, *Conogethes punctiferalis*, ginger, turmeric, mass production, infectivity.

Part - II: Investigator Profile

(Please identify clearly changes, if any in project personel)

810 Principal Investigator

8101	Name	: Dr. Rashid Pervez
8102	Designation	: Senior Scientist
8103	Division/Section	: Crop Protection/Nematology
8104	Location	: Kozhikode
8105	Institute Address	: Indian Institute of Spices Research, Kozhikode

811 Co-investigator

8111	Name	: Dr. Santhosh J. Eapen
8112	Designation	: Senior Scientist
8113	Division/Section	: Crop Protection/Nematology
8114	Location	: Kozhikode
8115	Institute Address	: Indian Institute of Spices Research, Kozhikode

812 Co-investigator

8121	Name	: Dr. S. Devasahayam
8122	Designation	: Principal Scientist & Head
8123	Division/Section	: Crop Protection/Nematology
8124	Location	: Kozhikode
8125	Institute Address	: Indian Institute of Spices Research, Kozhikode

Part - III: Technical Details

820 Introduction and Objectives

8201 Project objectives:

- Survey and documentation of EPNs in major ginger and turmeric growing areas.
- To identify most virulent strain/species of entomopathogenic nematodes against insect pests infesting ginger and turmeric.
- Standardization and development of mass production techniques of EPN.

8202 Background information and importance of the project

The shoot borer is highly polyphagous and has been recorded on 65 host plants including ginger and turmeric belonging to 30 families. The larvae of shoot borer bore in to the pseudostem and feed on the growing shoot of plant, resulting in yellowing and drying of infested pseudostems. The presence of bore holes on the pseudostem, through which frass is extruded, and the withered central shoot are characteristic symptoms of pest infestation.

Several methods are available to manage the shoot borer of ginger through chemical, cultural and integrated means. But these methods are not sufficient to minimize the losses due to this insect. Use of insecticides result in resistance, residue and resurgence, besides being highly costly. EPNs have been found to work effectively in other cropping systems especially vegetables, pulses and plantation crops for managing insect pests.

The project was envisaged to search and identify entomopathogenic nematodes (EPNs), which can survive in spice ecosystems and kill the target pests quickly. EPNs have broad host range and do not harm on non target organisms. Method of culture and technique of multiplication of EPN is not well understood. Through this project these problems will be tackled so that effective and economical control is achieved. Through this effort an effective EPN will be identified, its infectivity and mass production will be standardized.

821 Project Technical Profile:

8211 Technical Programme:

(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environment etc.)

Survey and collection of soil samples for detection of EPN

Survey and collected soil samples from ginger and turmeric rhizosphere from different locations of Kozhikode, Wyanad, Kottayam, Idukki (Kerala), Kodagu (Karnataka), Coimbatore (Tamil Nadu), Guwahati (Assam), Faizabad (Uttar Pradesh) and Barapani (Meghalaya) districts. About 250 g moist soil were collected from the rhizosphere of ginger at a depth of 10-20 cm in each site, each sample containing a composite of 5 random sub samples. Samples were placed in polyethylene bags to prevent moisture loss and a metal label providing information on date of collection, exact locality and host variety were included.

Isolation of EPNs

The composite soil (about 250 g) was placed in a plastic container. Five live *G. mellonella* larvae were used to bait out the EPNs, if any. The soil sample was checked every day for 7 days for the presence of EPNs. The dead larvae, if found, were placed in modified white trap for 2 weeks at room temperature for emergence of EPN.

Maintenance of EPNs and insect cultures

All isolated strains of entomopathogenic nematodes were maintained on final instar larvae of *G. mellonella* (Fig. 1). EPNs were multiplied using the methods of Woodring & Kaya (1988). Host insect *viz*. greater wax moth (*G. mellonella*) and rice moth (*Corcyra cephalonica*) were multiplied on artificial diet.

Collection of infected pseudostems of ginger for detection of EPN

Infected pseudostems of ginger with shoot borer larvae were collected from different localities. Pseudostems were placed in polyethylene bags and a metal label providing information on date of collection, exact locality and host variety was included.

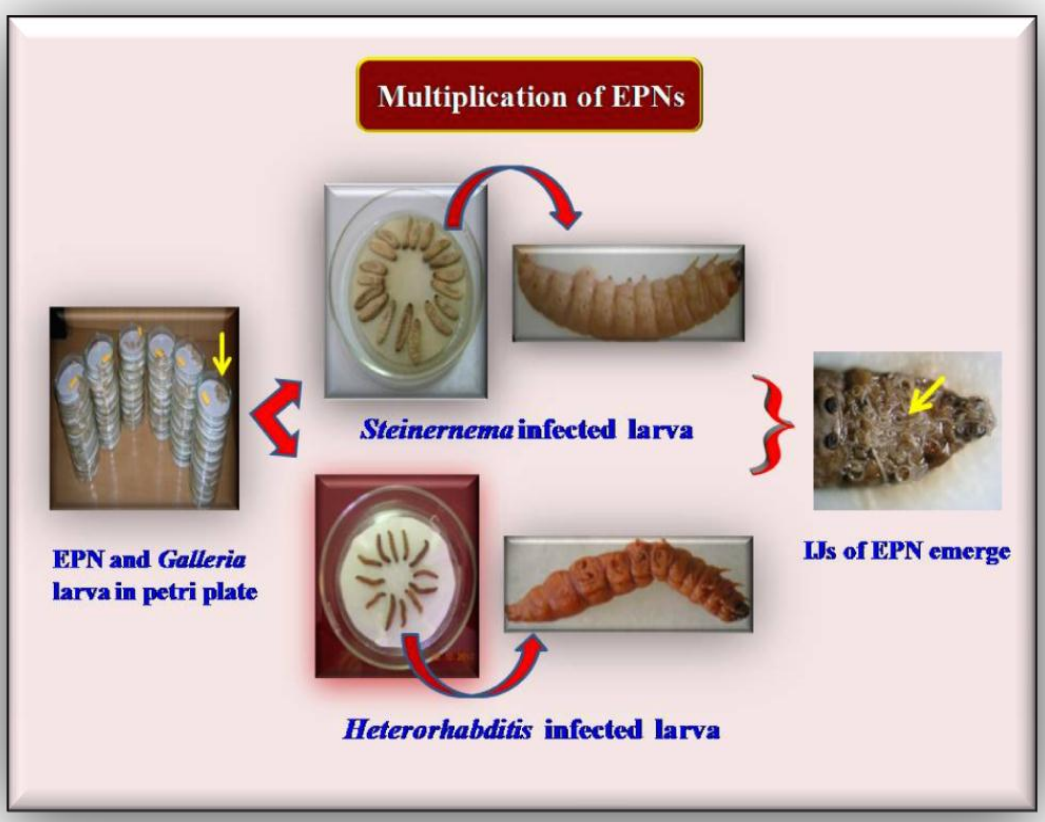

Fig. 1: Multiplication of EPNs on *G. mellonella* larva

Identification of EPN

***Morphometric studies*:** Nematodes were killed in warm water at 60 ºC and fixed in Triethnolamine formaldehyde (TAF) and kept in this solution for 48 h. Fixed nematodes were transferred into glycerine-alcohol solution for dehydration according to Seinhorst rapid method and male, female and infective juveniles (IJs) were mounted on glass slide using cover glass and glass rod supports to avoid flattening.

***Light microscopy*:** Light microscope photographs of males, females and IJs were taken by Leica microscope at different magnifications.

Measurements: Holotype male, 10 paratype each of males, females and infective juveniles were measured using an ocular micrometer. Selection of morphometric characters was done according to Hominick *et al.* (1997).

Line diagrams: Illustrations were prepared with the help of armed type camera lucida.

Scanning Electron Microscopy (SEM): Adults and IJs were fixed in 2.5% glutaraldehyde buffer with 0.1 M sodium cacodylate at pH 7.2 for 24 h at 8 ^{0}C. Fixed nematodes were rinsed in distilled water three times. They were post fixed with 2% osmium tetraoxide solution for 12 h at 25 ^{0}C, rinsed in distilled water three times again and dehydrated in 30, 50, 70, 90 and 100% ethanol at 30 min intervals. They were then critical point dried with liquid CO_2, mounted on SEM stubs, and coated with gold (Nguyen and Smart, 1995). Photographs were taken by scanning electron microscopy (LEO435 UP).

Entomopathogenicity: Entomopathogenicity of *Steinernema ramanai* sp. n. and *Oscheius gingeri* sp. n. were evaluated against *G. mellonella* larva by petri dish method. Experiments were conducted at room temperature along with control and replicated five times. Observations on mortality of insects were recorded after 24 h interval.

Efficacy of EPNs against insect pests infesting ginger

Nematode and insect cultures*:* Eight test EPNs, *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02), *S. ramanai* sp. n., *S. carpocapsae O. gingeri* sp. n. and *Oscheius* spp. (IISR 04, 05 and 08) were utilized for the study. The test insect, *C. punctiferalis* larvae were collected from ginger fields of IISR Experimental Farm, Peruvannamuzhi and hairy caterpillar larva, *Euproticis* sp. from farmer's field in Thamarassary, Kozhikode District. The larvae were sorted out and those of same size were utilized for the study.

Efficacy of EPNs**:** Efficacy of EPNs against *Euprotis* sp. and *C. punctiferalis* larvae was tested in six well plates (Fig. 2). One larva of test insect was kept in each well and 100 IJs of each test species of EPN were inoculated and their mortality was recorded after 72 h. The experiment was conducted at 28 ^{0}C in a BOD incubator and

replicated 20 and 12 times, respectively, along with control. The mortality was calculated according to following formula:

$$\text{Mortality (\%)} = D \times 100 / N$$

Where:

D- Number of dead larvae; N – Total number of larvae

Efficacy of EPNs against shoot borer pupae was tested in plastic containers (Fig. 3). One pupa/container was kept and 500 IJs of tested species of EPN in 0.5ml water were released in each container and observations on mortality were recorded after 7 days. All experiments were conducted at room temperature and replicated six times along with control (water only).

Multiplication of EPNs: EPN infected dead larvae of test insects were removed from the well and kept on White trap for emergence of EPNs from the body of insect. IJs were collected daily, till the emergence stopped in about 15 days. From this collection, the total emerged populations of EPNs were counted thrice under a stereoscopic binocular microscope with the help of Syracuse counting dish and mean values were worked out.

Rate of IJs penetration into host: The penetration of IJs into shoot borer larva was tested in petri plates. One larvae was kept in petri plate and each EPN species at a concentration of 500 IJs in 0.5 ml water was sprayed over filter paper placed at the bottom of the petri plate. The petri plate was kept at 28 ^{0}C for 72 h and replicated six times. After that, the enzymatic digestion of dead larvae was done using 3 ml pepsin in a tube and kept in a shaker incubator at 120 rpm for 1 h. The tubes were then shaken well and returned to the shaker for another 20 min after which 7 ml of tween 80 were added to each tube and shaken very well and kept at 5 ^{0}C for 48 h or until the nematode count was done. The penetration rate was calculated according to following formula:

$$PR = N_1 \times 100/N_2$$

Where:

PR - Penetration rate, N_1 - Mean nematode number found within host and N_2 - Original nematode number used.

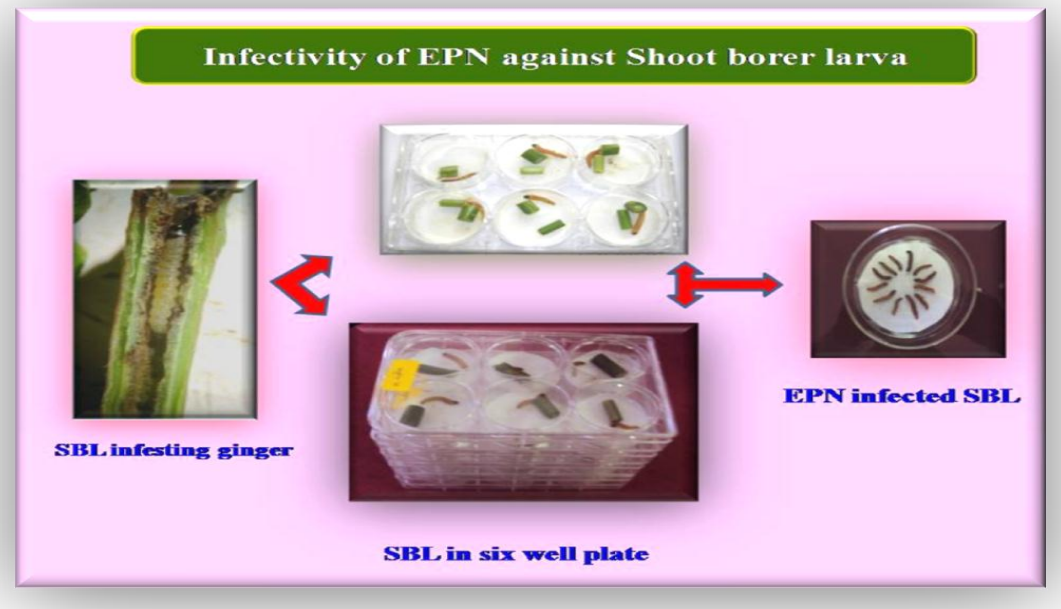

Fig. 2: Infectivity of EPNs tested against shoot borer larava

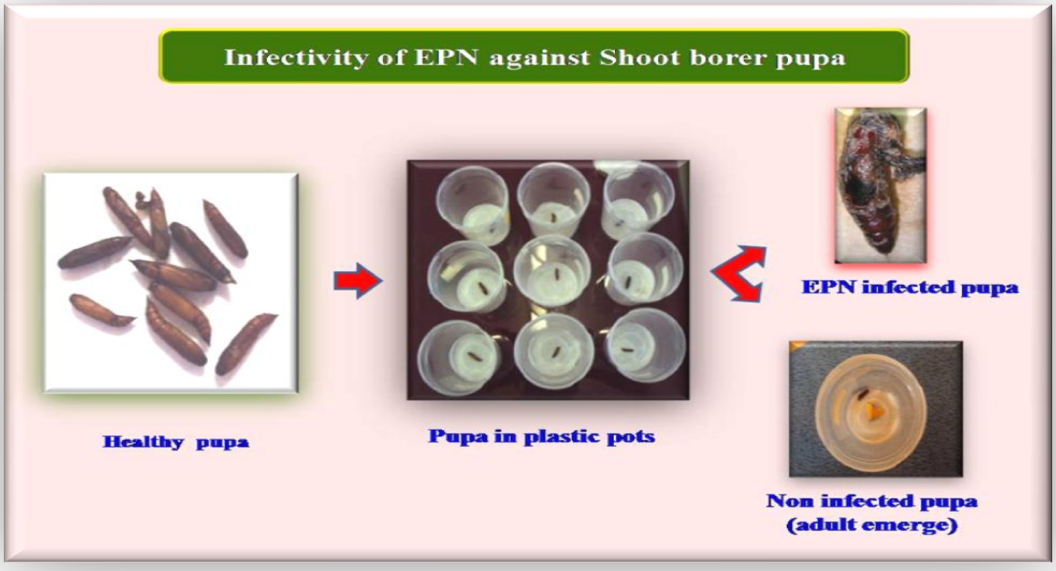

Fig. 3: Infectivity of EPNs tested against pupa of shoot borer

Infectivity of NBAII isolate of EPNs

Nematode and insect cultures: Two EPNs, *Heterorhabditis indica* (NBAII Hi 01) and *Steinernema abbasi* (NBAII Sa 01) were obtained from National Bureau of Agricultural Important Insect (NBAII), Bangalore. Both EPN species were cultured on fully grown *G. mellonella* larvae. The IJs were surface sterilised in 0.1% Hyamine solution for 2 minutes then washed thrice in sterilised distilled water and stored in distilled water in tissue culture flasks for study. The shoot borer larvae were collected from ginger fields of the IISR Experimental Farm, Peruvannamuzhi. The larvae were sorted out and those of same size were used for the study.

Infectivity of EPNs: The efficacy of EPNs against shoot borer, *C. punctiferalis* larva was tested in six well plates (3.5 cm diameter) lined with filter paper at the bottom of the plate. For this, one larva of the test insect was kept in each well and 100 IJs of each test species of EPN in 0.5 ml water were inoculated and their mortality was recorded after 48 h. The experiment was conducted at 28 °C in a BOD incubator and replicated 12 times along with control. The per cent mortality and mean values were determined.

The efficacy of EPNs was tested against shoot borer pupae in plastic containers (5.5 cm diameter) lined with filter paper at the bottom of the containers. One pupa/container was kept and 500 IJs of test species of EPN in 0.5 ml water were released in each container and observations on their mortality were recorded after 7 days. The experiments were conducted at room temperature and each treatment was replicated six times along with control (water only).

Multiplication of EPNs**:** EPN infected dead larvae of test insects were removed from the well and kept on the White trap for emergence of infective juveniles (IJs) of EPNs from the body of the insect. IJs were collected daily, till the emergence stopped in about 15 days. From this collection, the total emerged populations of EPNs were counted thrice under a stereoscopic binocular microscope, with the help of Syracuse counting dish, and mean values were determined.

Effect of temperatures on infectivity and multiplication of EPNs

***Nematode and insect cultures*:** All eight EPNs, *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02), *S. ramanai* sp. n., *S. carpocapsae O. gingeri* sp. n. and *Oscheius* spp. (IISR 04, 05 and 08) were obtained from the Nematology Laboratory of this institute and test insect, *C. punctiferalis* larvae were collected from ginger fields of IISR Experimental Farm, P'muzhi and farmers fields Kodagu district (Karnataka). The larvae were sorted out and those of same size were utilized for the study.

Bioassay: Infectivity of eight isolates of EPNs against shoot borer larva was tested in petri plates at different temperatures *viz.*, 20, 25, 28, 30 and 35 ^{0}C. For this, 10 larva of test insect was kept in each plate and 500 IJs of each test species of EPNs were inoculated and their mortality was recorded after 72 h. The experiment was replicated 10 times along with control. The mortality was calculated into percentages.

***Multiplication of EPNs*:** EPN infected dead shoot borer larvae of test insect were removed from the plate and kept on White trap at different temperatures *viz.*, 20, 25, 28, 30 and 35 ^{0}C for emergence of infective juveniles. IJs were collected daily, till the emergence stopped in about 15 days. From this collection, the total emerged populations of EPNs were counted thrice under a stereoscopic binocular microscope with the help of Syracuse counting dish and mean values were worked out.

Mass production of EPNs

Mass *in vitro* production of eight isolates of EPNs, *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02), *S. ramanai* sp. n., *S. carpocapsae O. gingeri* sp. n. and *Oscheius* spp. (IISR 04, 05 and 08) was evaluated in various media *viz.*, Wout's, wheat flour, dog biscuit, egg yolk, nutrient agar and agar-agar (Table 1).

Sponge, which served as a carrier material, was cut into small pieces of 1 cm size and washed with 2% formaldehyde, then rinsed 2-3 times with distilled water separately and dried under room temperature. The ingredients of each medium were mixed homogenously and coated to sponge pieces till they were soaked in the media. The sponge pieces were transferred carefully to the conical flask. The mouth of the conical flask was cleaned and plugged with non-absorbent cotton and autoclaved for 20 min at 121 ^{o}C.

Fresh IJs extracted were inoculated aseptically into the flasks @ 1000 IJs/flask under laminar flow. The sealed flasks were incubated at 30 ^{o}C for 25 days. Care was taken not to shake the flask after the inoculation of nematodes. The colonies of the EPN started appearing on the walls of the flasks after 20 days post inoculation (Fig. 4).

After 25 days of inoculation, IJs were harvested. The sponge chips were piled up on a 100 mesh sieve. The sieve was placed in a plastic pan of distilled water overnight. The nematodes moved out of the sponge into the water, passed through the sieve and accumulated on the bottom of the pan. The suspension were transferred into beaker and cleaned by Cobb's sieving and decanting method (Fig. 4). The nematodes thus collected and population of EPN in each media was counted three times with the help of Syracuse counting dish with the help of stereoscopic microscope and mean value was worked out.

Table 1: Ingredients of artificial media, evaluated for mass production of EPNs.

S. No.	Media	Ingredients
1.	Wout's	Nutrient broth (0.88 g), yeast extract (0.32 g), soybean flour (14.40 g), groundnut oil (10.40 g) and distilled water (60 ml)
2.	Wheat flour	Wheat flour (15 g), Chickpea flour (5 g), beef extract (5 g), yeast extract (6 g), agar 1% (1 g), coconut oil (6 g) and distilled water (60 ml)
3.	Egg yolk	Egg yolk (7 g), yeast extract (2 g), NaCl (0.8 g), oil (15 g) and distilled water (60 ml)
4.	Dog biscuit	Dog biscuit (15 g), yeast extract (1 g), peptone (3 g), agar (2 g), oil (10 g) and distilled water (60 ml)
5.	Nutrient agar	Nutrient agar (2 g), beef extract (2 g) and distilled water (200 ml)
6.	Agar agar	Agar agar (3 g), milk powder (0.5 gm) and distilled water (200 ml)

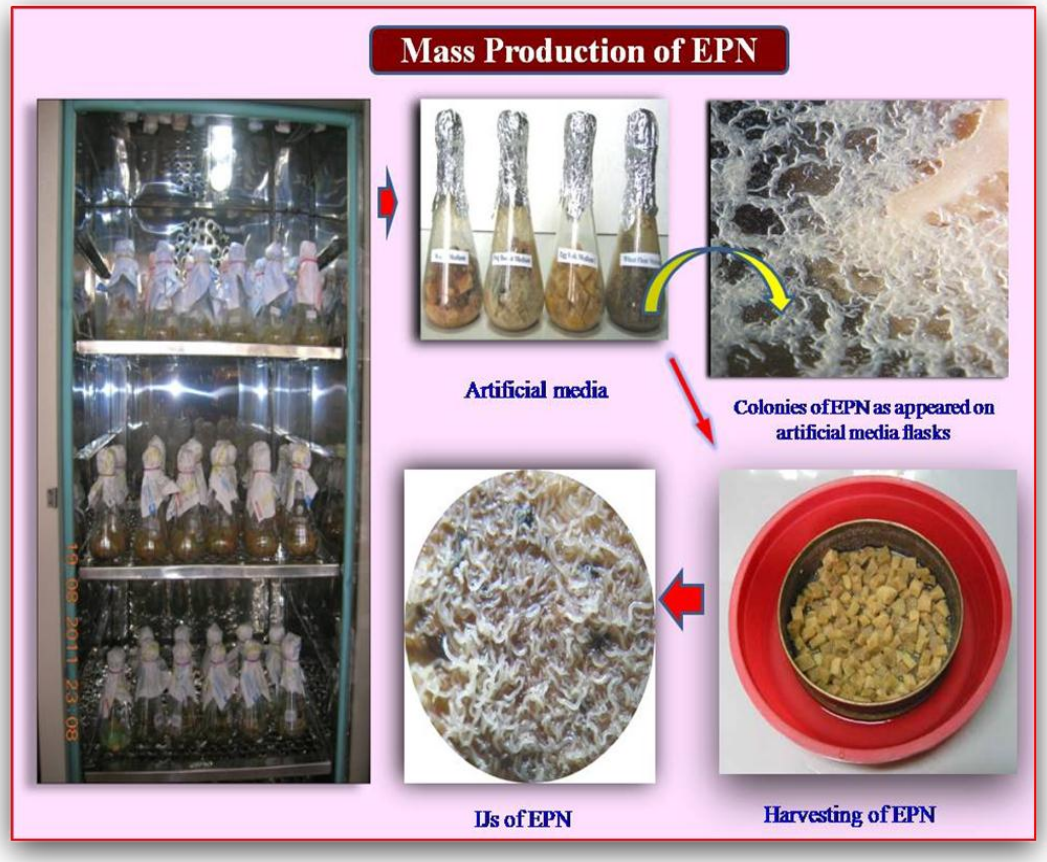

Fig. 4: Mass production of infective juveniles of EPNs in artificial media

8212 Total man months involvement of component project workers

a) Scientific **42 man months**

Rashid Pervez (18)

S. J. Eapen (12)

S. Devasahayam (12)

b) Technical **12 man months**

822 Final Report on the Project

Detailed report containing all relevant data with a summary of results

(not exceeding 2-5 pages)

8221 Achievements in terms of targets fixed for each activity

Survey and collection of soil samples for detection of EPNs

One hundred and fifty seven soil samples were collected from ginger and turmeric rhizosphere from different locations of Kozhikode, Wyanad, Kottayam, Idukki (Kerala), Kodagu (Karnataka), Coimbatore (Tamil Nadu), Guwahati (Assam), Faizabad (Uttar Pradesh) and Barapani (Meghalaya) districts.

Isolation of EPNs through baiting method

Out of 154 soil samples baited out, only eight samples were found to be positive to entomopathogenic nematodes. Among these EPN strains, three EPNs were found from Kozhikode district, three from Idduki district and one each from Waynand and Faizabad districts (Table 2).

Collection of infected pseudostems of ginger for detection of EPNs

Four hundred and twenty four shoot borer larvae were collected from IISR experimental farm, Perunnamuzhi and different localities of Wyanand and Kodagu districts. Out of these larvae, 112 larvae were found dead. Among these larvae, 11 associated with mermethids, 8 with rhabditids nematodes and only one was EPN (Table 3).

Maintenance of EPNs and host insect culture

All eight isolated strains of entomopathogenic nematodes were maintained using final instar larvae of *G. mellonella*. Nematodes were multiplied using the methods of Woodring & Kaya (1988). Freshly emerged IJs were surface sterilised in 0.1% hyamine solution and washed three times in distilled water and stored at 15 ^{o}C in distilled water for further use.

Host insect *G. mellonella* were multiplied on artificial diet (Table 4).

Yeast tablets were grinded into a fine powder and mixed with corn flour, wheat bran and milk powder (Part A). Glycerin and honey were mixed separately (Part B). Finally part A and B were mixed together thoroughly and a homogenous mixture was prepared. The content of artificial diet was distributed in two plastic containers (5 l capacity).

Eggs of *G. mellonella* larvae were released in each container and incubated at room temperature. The larvae were ready for use within 3 weeks. After 2-3 weeks, the larvae were drawn for multiplication of EPN.

Table 3: Shoot borer larvae collected for the detection of EPNs

Location	**No. of larvae collected**	**No. of dead larvae**	**No. of larvae infested with nematodes**
1. Kozhikode			
IISR Farm, Peruvannamuzhi	256	77	18 (9 mermithids, 8 rhabditids, 1 EPN)
2. Wyanand			
Kalpetta	76	12	-
Muthoviddi	8	8	-
Karimbummel	14	3	-
3. Kodagu			
Gonnikopal	19	-	-
Madikeri	51	12	2 (2 mermithids)
Total	**424**	**112**	**20 (11 mermithids, 8 rhabditids, 1 EPN)**

Table 2: Soil sample analysis for detection of EPNs.

Location	No. of samples collected	Positive samples	EPN baited out
1. Kozhikode			
IISR Farm, Peruvannamuzhi	40	03	*Heterorhabditis* sp. (IISR 01) *Steinernema* sp. (IISR 02) *Oscheius gingeri* sp. n.
2. Wyanad			
A. Vythiri			
Kalpetta	05	-	-
Chundale	03	-	-
Thariode	02	-	-
Muttil	03	-	-
B. **Manathavady**			
Porunnannur	03	-	-
Payyappally	03	-	-
Thirunelly	03	-	-
C. **Sulthan Bathery**			
Ambalavayal	16	01	*Oscheius* sp. (IISR 05)
Poothadi	01	-	-
Pulpally	06	-	-
3. Idukki			
Udumbanchola	18	01	*S. carpocapsae*
Adimali	7	01	*S. ramanai* sp. n.
Peerumedu	12	01	*Oscheius* sp. (IISR 04),
Devikulam	5	-	-
4. Kottayam	4	-	-
5. Guwahati (Assam)	5	-	-
6. Coimbatore (TN)	3	-	-
7. Barapani (Meghalaya)	7	-	-
8. Faizabad (UP)	8	01	*Oscheius* sp. (IISR 08)
Total	**154**	**8**	

Table 4: Ingredients of artificial diet for culture of *G. mellonella*

	Ingredients	Qty.
Part - A	Corn flour	200 g
	Wheat bran	100 g
	Skimmed milk powder	100 g
	Yeast tablets	50 g
Part-B	Glycerin	100 ml
	Honey	100 ml

Some of the *G. mellonella* larvae were left to complete their life cycle and emergence of moth. These moths were collected and placed in a separate jar in which several small pieces of honey combs were provided with hanging folded paper strips which served as dark areas for hiding and substrate for egg laying. The neonate larvae in honey combs or eggs collected from paper strips were put on artificial diet.

Identification of EPNs

Out of eight isolated species of EPN, two species belonged to genus *Steinernema*; one to *Heterorhabditis* and five to *Oscheius*. Among them, two new species *viz., Steinernema ramani* sp. n. and *Oscheius gingeri* sp. n. were identified on the basis of morphometric and molecular characterization.

Steinernema ramanai sp. n.

Measurements of Steinernema ramanai sp. n. **:**Measurements of various characters of the holotype male, paratype males, females and infective juveniles of *S. ramanai* sp. n. are given in Table 5.

Type host and locality**:** The natural host of *S. ramanai* sp. n. is unknown. The nematode was trapped by baiting with *G. mellonella* from the soil. Soil samples were obtained from rhizosphere of ginger cultivated at Indian Institute of Spices Research Experimental Farm, Peruvannamuzhi, Kozhikode District (11º 35’52.34” N, 75º 49’ 20.48” E, elev 58 m), Kerala, India.

***Type material*:** Holotype male, 10 paratypes each of females, males and infective juveniles were deposited in Nematology Laboratory, Indian Institute of Spices Research, Calicut.

***Etymology* :** This species is named after Dr. K. V. Ramanna, former nematologist and Assistant Director General (Plantation crops), Indian Council of Agricultural Research, New Delhi, India

Morphological diagnosis and relationships: *S. ramanai* sp. n. is characterised by the length of the infective juvenile (377µm) which is the smallest nematode among the *Steinernema* species, presence of five incisures in the lateral field, elongate conoid tail, gradually tapering at tip; female 822 µm long, vulva with double flapped epipytigma and tail without post anal swelling; males 672 µm long, body 'J' shaped upon fixation, presence of 10 genital papillae, mucron present on the tail terminus.

***Entomopathogenicity*:** *S. ramani* sp. n. was pathogenic to *G. mellonella* larva and started killing the larvae within 24 h (36 %), whereas, it brought about cent percent mortality within 72 h post exposure. Significant and positive correlation was found between mortality of insect and observation periods (R^2 = 0.839) (Fig. 5).

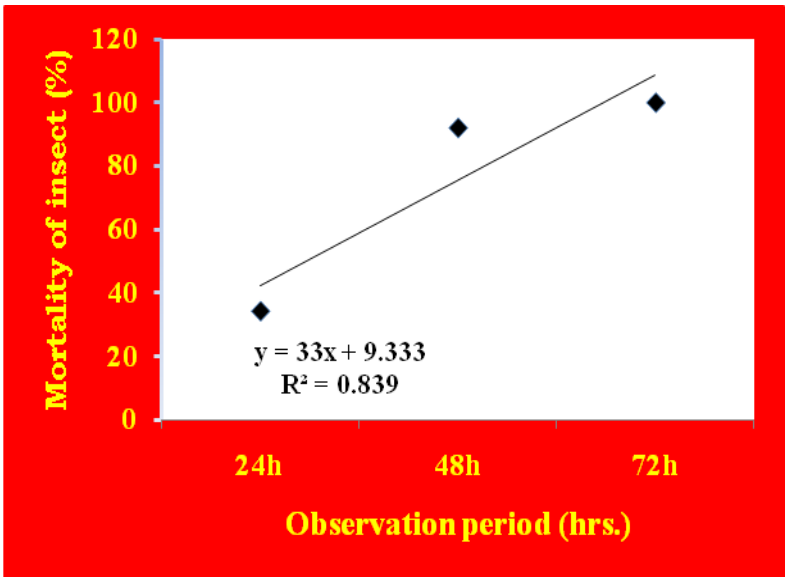

Fig. 5: Pathogenicity of *S. ramani* sp. n. against *G. mellonella* larvae

Table 5. Morphometrics of *Steinernema ramanai* sp. n. (All measurements are in µm and in the form of mean (range)

Character	**Holotype (Male)**	**Infective Juvenile**	**Male**	**Female**
N		**10**	**10**	**10**
Total length	646.99	377.0 (334.6-421.1)	672.5 (646.9-685.7)	822.2 (777.1–866.2)
Greatest width	38.81	21.3 (20.3-23.2)	43.6 (38.8 – 48.5)	63.3 (56.2 – 71.7)
Stoma length	12.23	5.4 (4.8-5.8)	13.1 (12.6 – 13.5)	14.4 (13.5 – 14.5)
Stoma width	10.67	-	11.3 (10.6 – 11.6)	12.2 (9.7 – 13.5)
EP	49.47	34.5 (32.0 – 35.8)	50.7 (49.4 – 52.3)	34.5 (29.1 – 41.7)
EPW	23.28	14.1 (13.5-14.5)	24.2 (23.2 – 25.2)	19.4 (18.6-26.7)
ES	126.10	77.4 (69.8 – 89.2)	133.5 (126.1–138.7)	147.1 (140.6– 150.3)
ABD	24.25	12.6 (10.67 – 13.58)	23.6 (18.4 – 28.1)	24.5 (24.2 – 25.2)
Tail	19.93	42.0 (37.8 – 44.6)	18.9 (16.4 – 20.3)	37.8 (36.86 – 39.77)
Spicule length (SL)	53.35	-	53.9 (50.4 – 58.2)	-
Gubernaculam length	29.41	-	28.8 (28.1 – 29.4)	-
Anterior to vulva	-	-	-	483.7 (447.7 – 514.1)
Vulva to anus distance	-	-	-	300.7 (291.0 – 315.2)
a	16.67	17.7 (14.3 - 20.1)	15.4 (14.1 – 16.6)	12.9 (11.4 – 13.9)
b	5.13	4.8 (4.7 – 5.1)	5.0 (4.9 – 5.1)	5.5 (5.4 – 6.1)
c	32.46	8.9 (7.6 – 9.9)	35.6 (33.3 – 41.5)	21.7 (19.5 – 23.5)
c’	0.82	3.3 (3.2 – 3.5)	0.83 (0.58 – 1.10)	1.5 (1.4 – 1.6)
V (%)	-	-	-	58.7 (57.9 – 59.3)
D (%)	39.2	44.5 (40.2 – 51.4)	37.9 (36.3 – 39.2)	23.4 (20.6 – 27.7)
E (%)	248.21	82.1 (80.4 – 84.6)	268.2 (247.6 – 317.6)	91.2 (78.9 – 104.8)
SW (SL/ABD)	2.20	-	2.3 (2.1 – 2.8)	-

Oscheius gingeri sp. n.

***Measurements of O. gingeri sp. n.*:** Measurements of various characters of the holotype male, paratype males, females and infective juveniles of *O. gingeri* sp. n. are given in Table 6.

***Type host*:** The natural host of *O. gingeri* sp. n. is unknown. The nematode was trapped by baiting with *G. mellonella* from the soil.

***Type locality*:** Soil samples were obtained from rhizosphere of ginger cultivated at Indian Institute of Spices Research Experimental Farm, Peruvannamuzhi, Kozhikode District (11º 35'52.34" N, 75º 49' 20.48" E, elev 58 m), Kerala, India.

***Etymology*:** This species is named after its isolation from ginger (*Zingiber officinale* Rosc.) rhizoshphere.

***Morphological diagnosis and relationships*:** The leptoderan bursa and crochet needle shaped spicules place *O. gingeri* sp. n. in the insectivora group of *Oscheius* (Sudhaus & Hooper, 1994; Sudhaus & Fitch, 2001). *O. gingeri* sp. n. is characterised by the length of the male (739 µm) which is the smallest nematode among the *Oscheius* species of insectivora group, presence of leptoderan bursa and crochet needle shaped spicules, presence of 10 incisures in the lateral field, didelphic female reproductive system, double flapped epipytigma present on the vulval opening and presence of nine genital papillae.

***Entomopathogenicity*:** *O. gingeri* sp. n. was pathogenic to *G. mellonella* larva and started killing the larvae within 24 h (34 %), whereas, it brought about cent per cent mortality within 72 h post exposure. Significance correlation was found between mortality of insect and observation periods ($R^2 = 0.83$) (Fig. 6).

Efficacy of EPNs against insect pests infesting ginger

There was no significant difference in hairy caterpillar (*Euprotis* sp.) larva mortality among the EPN isolates. Out of the eight tested EPN isolates, seven isolates caused 100% mortality of hairy caterpillar and only *Oscheius* sp. (IISR 08) caused less (almost 95%) mortality (Fig. 7).

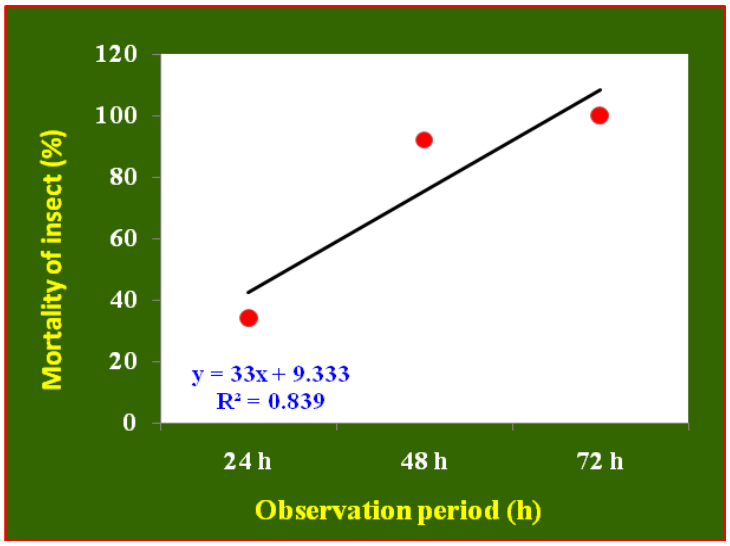

Fig. 6: Pathogenicity of *O. gingeri* sp. n. against *G. mellonella* larvae

All the tested EPN isolates caused mortality of shoot borer (*C. punctiferalis*) larvae, but the level of mortality varied significantly ($P < 0.05$) between the EPN isolates. Among the tested EPN isolates, *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 07 and 08) caused 100% mortality to shoot borer larvae, while *Steinernema* sp. (IISR 03) and *Oscheius* spp. (IISR 04 and 05) caused 92% and *S. carpocapsae* 83% mortality (Fig. 8). There was no mortality of shoot borer or hairy caterpillar larvae in the controls after 72 h.

***Multiplication of EPNs*:** Almost all the EPN isolates multiplied on the tested insects, but the level of multiplication varied significantly between EPN isolates. Larvae of the shoot borer were better hosts ($P < 0.05$) than those of hairy caterpillar for the multiplication of IJs of the EPNs.

In the shoot borer larvae (Table 7), the greatest number (82,986 IJs/larva) of infective juveniles was observed for *Steinernema* sp. (IISR 02), followed by *Oscheius* sp. (IISR 05) (73,187 IJs/larva), which were at par, and the fewest IJs were observed for *Steinernema* sp. (IISR 03) and *Oscheius* sp. (IISR 07) (10,432 and 14,373 IJs/larva, respectively). *Oscheius* sp. (IISR 05), *Heterorhabditis* sp. (IISR 01) and *S. carpocapsae* showed intermediate levels of multiplication.

Table 6: Morphometrics of *O. gingeri* sp. n. (All measurements are in µm and in the form of mean (range)).

Character	Holotype (Male)	Infective Juvenile	Paratype	
			Female	Male
N		**10**	**10**	**10**
Total length	678.1	442.1 (331.1-520.2)	1638.6 (1418.3-1813.1)	739.5 (673.1-821.5)
Greatest width	32.6	17.2 (16.23-18.2)	81.6 (75.1-89.1)	35.2 (31.6-38.9)
Stoma length	17.9	14.4 (13.9-15.2)	19.5 (18.7-20.8)	17.9 (16.8-19.2)
Stoma width	3.6	1.7 (1.61-2.13)	3.1 (2.99-3.2)	3.6 (3.1-3.9)
EP	116.7	92.3 (87.36-96.17)	204.1 (187.1-223.2)	126.1 (110.2-141.8)
EPW	31.6	17.2 (15.3-18.9)	51.2 (49.16-53.16)	32.2 (30.5-33.6)
NR	110.8	68.9 (60.1-74.1)	195.8 (178.1-203.1)	105.2 (90.5-114.3)
ES	171.7	95.9 (83.1-101.5)	253.2 (189.1-283.5)	171.4 (142.5-187.5)
Testis ratio	10.8	-	-	10.8 (10.1-12.2)
Vulval opening	-	-	54.1 (51.3-60.2)	-
ABW	16.8	12.1 (10.1-14.2)	26.4 (25.2-28.1)	17.7 (15.5-19.2)
Tail	45.8	52.1 (43.5-60.1)	121.3±5.31 (115.2-129.1)	51.8 (43.5-59.3)
SL	24.6	-	-	25.7±1.31 (24.1-27.3)
GL	9.6	-	-	9.3±0.56 (8.69-9.86)
a	19.8	24.6 (22.81-27.18)	20.3 (18.5-21.23)	20.6 (18.3-24.0)
b	4.5	4.3 (3.61-5.1)	5.2 (5.15-5.35)	4.7 (4.3-5.3)
c	16.1	7.2 (6.4-7.9)	12.8 (12.1-13.2)	13.6 (11.5-16.7)
SW	1.6	-	-	1.4 (1.3-1.6)
D (%)	67.9	96.8 (88.9-115.6)	74.9 (64.7-81.7)	74.2 (61.1-90.4)
E (%)	272.6	179.6 (88.9-115.6)	168.5 (151.1-193.8)	247.8 (191.9-312.2)

In the larvae of hairy caterpillar, significantly largest number of IJs (33,750/larva) was recorded for *Oscheius* sp. (IISR 08), followed by *Steinernema* sp. (IISR 03) and *Oscheius* sp. (IISR 05), which had the same number (15,600/larva). The reproduction of the remaining EPNs was significantly much less. No multiplication of *Oscheius* sp. (IISR 04) was recorded in hairy caterpillar (Table 7).

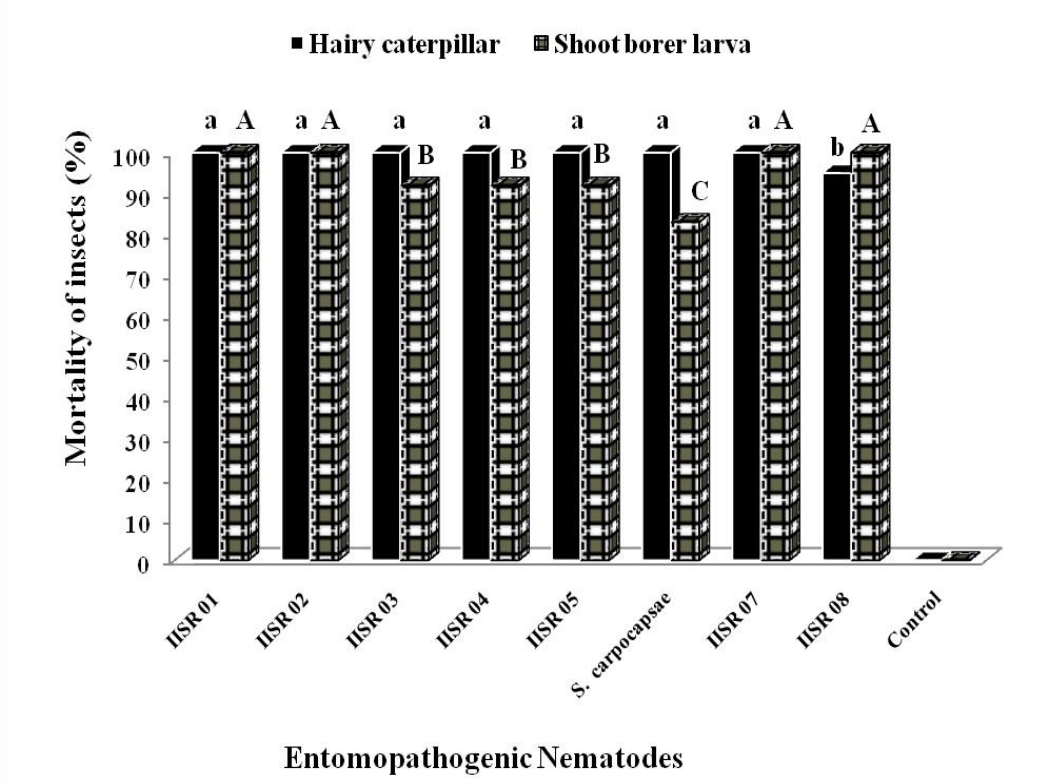

Fig. 7. Mortality of hairy caterpillar (*Euproctis* sp.) and shoot borer (*Conogethes punctiferalis*) larvae caused by IJs of different isolates of EPNs. Bars with different letters indicate significant differences according to Duncan's multiple range test at P = 0.05. Lower case letters for hairy caterpillar larvae and capital letters for shoot borer larvae.

***Efficacy of EPNs against pupae of shoot borer* :** There were significant differences (P <0.05) in the mortality of shoot borer pupa with the various EPNs. Among the EPNs tested, *Oscheius* sp. (IISR 07) was the most virulent isolate against the shoot borer pupae, causing 100% mortality, followed by 67% mortality by *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 05). No mortality was recorded with *Steinernema* sp. (IISR 03) and *Oscheius* sp. (IISR 04 and 08) (Fig. 8).

***Number of IJs penetrating into larvae of shoot borer*:** The numbers of IJs of different isolates of the tested EPNs that penetrated into the body of the shoot borer larvae showed significant differences ($P < 0.05$). Among the tested species, the largest number of IJs that penetrated into shoot borer larvae (15.5 IJs/larva) were of *Steinernema* sp. (IISR 03), followed by *Heterorhabditis* sp. (IISR 01) (10.7 IJs/larva), whereas a significantly smaller number (2.8 IJs/larva) was observed for *Oscheius* sp. (IISR 08) (Table 8).

Table 7. Multiplication of different isolates of EPNs on hairy caterpillar (*Euproctis* sp.) and shoot borer (*C. punctiferalis*) larvae.

EPN	No. of IJs/larva	
	Hairy caterpillar larva	**Shoot borer larva**
Heterorhabditis sp. (IISR 01)	1,720 de	59,020 cd
Steinernema sp. (IISR 02)	3,067 cde	82,987 a
Steinernema sp. (IISR 03)	15,600 b	10,432 f
Oscheius sp. (IISR 04)	-	73,817 ab
Oscheius sp. (IISR 05)	15,600 b	68,200 bc
Steinernema carpocapsae	5,460 c	51,086 d
Oscheius sp. (IISR 07)	4,740 cd	14,373 f
Oscheius sp. (IISR 08)	33,750 a	35,298 e

Mean values, in the same column, followed by different letter are significantly different according to Duncan's multiple range test at $P = 0.05$.

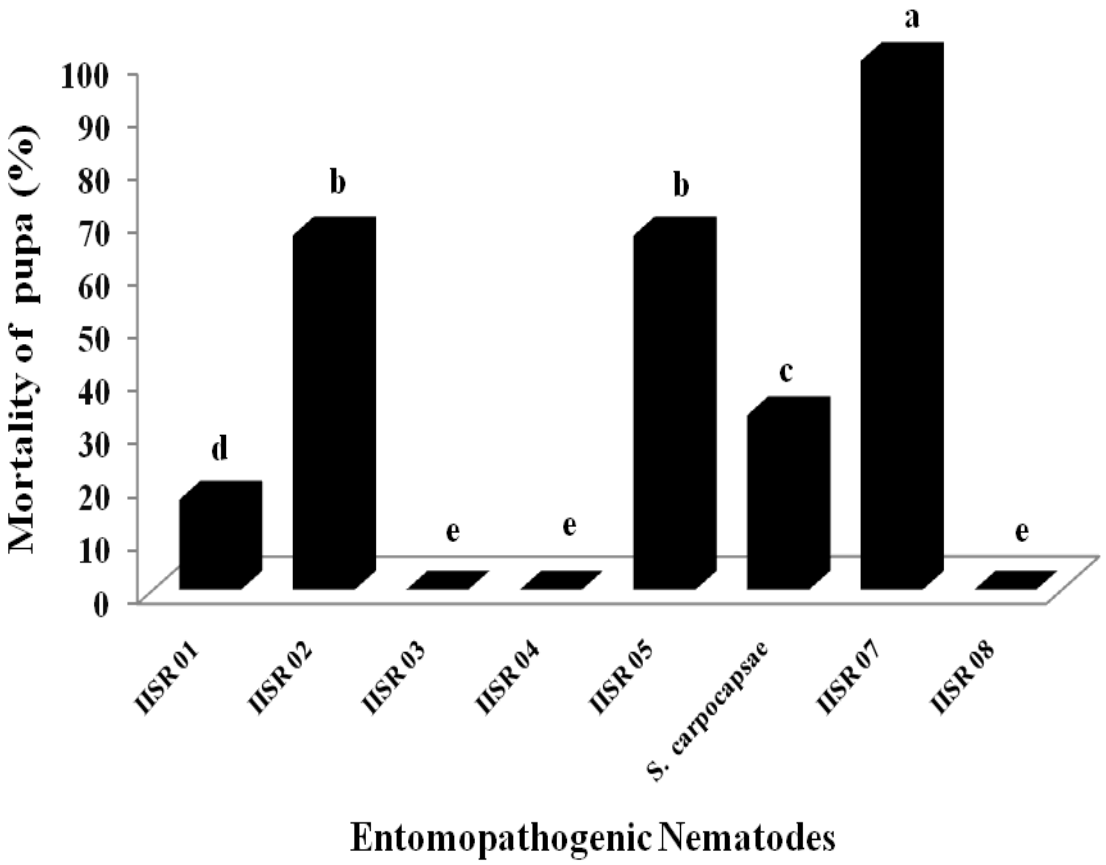

Fig. 8. Mortality of shoot borer (*C. punctiferalis*) pupae caused by IJs of different isolates of EPNs. Bars with different letters indicate significant differences according to Duncan's multiple range test at P = 0.05.

Table 8. Number of IJs of different isolates of EPNs penetrating into shoot borer larvae.

EPN	No. of IJs/larva
Heterorhabditis sp. (IISR 01)	10.7 ab
Steinernema sp. (IISR 02)	8.2 bcd
Steinernema sp. (IISR 03)	15.5 a
Oscheius sp. (IISR 04)	4.3 cd
Oscheius sp. (IISR 05)	4.8 cd
Steinernema carpocapsae	9.3 bc
Oscheius sp. (IISR 07)	6.0 bcd
Oscheius sp. (IISR 08)	2.8 d

Mean values followed by different letters are significantly different according to Duncan's multiple range test at P = 0.05.

Infectivity and multiplication of NBAII EPNs against shoot borer larva and pupa

Both species *S. abbasi* (NBAII Sa 01) and *H. indica* (NBAII Hi 01) were found pathogenic against shoot borer larvae and it brought about cent per cent mortality within 48 h, whereas, only 17 and 33 % mortality, respectively was found against shoot borer pupa (Fig. 9).

In case of multiplication of IJs of tested EPNs from shoot borer larva, both EPN isolates multiplied on the tested insect, but the level of multiplication varied significantly (Fig. 10). Maximum number (18,277 IJs/ cadaver) of infective juveniles of *S. abbasi* (NBAII Sa 01), whereas the lesser number juveniles (5135 IJs/ cadaver) of *H. indica* (NBAII Hi 01) multiplied on shoot borer larva.

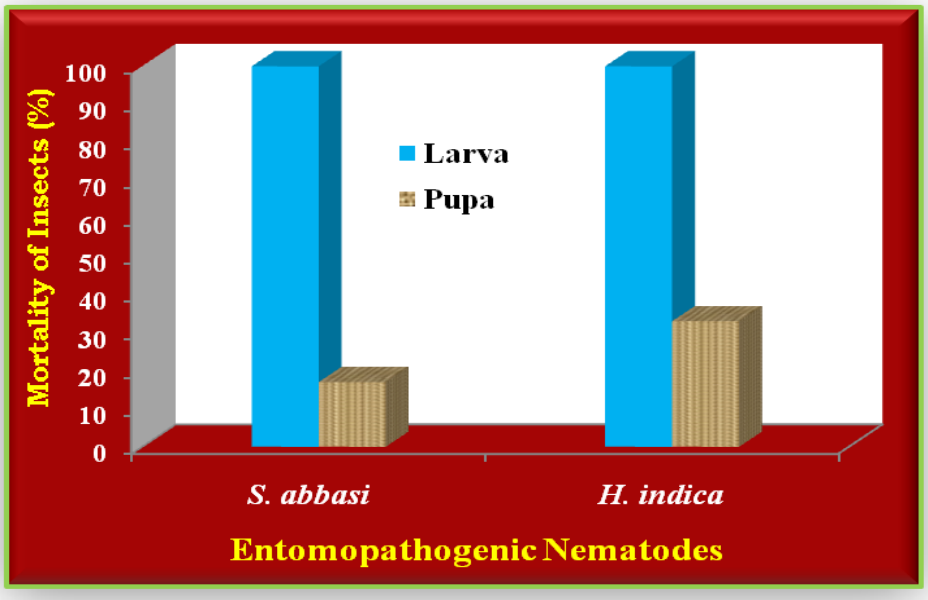

Fig. 9: Efficacy of entomopathogenic nematodes against shoot borer larva and pupa.

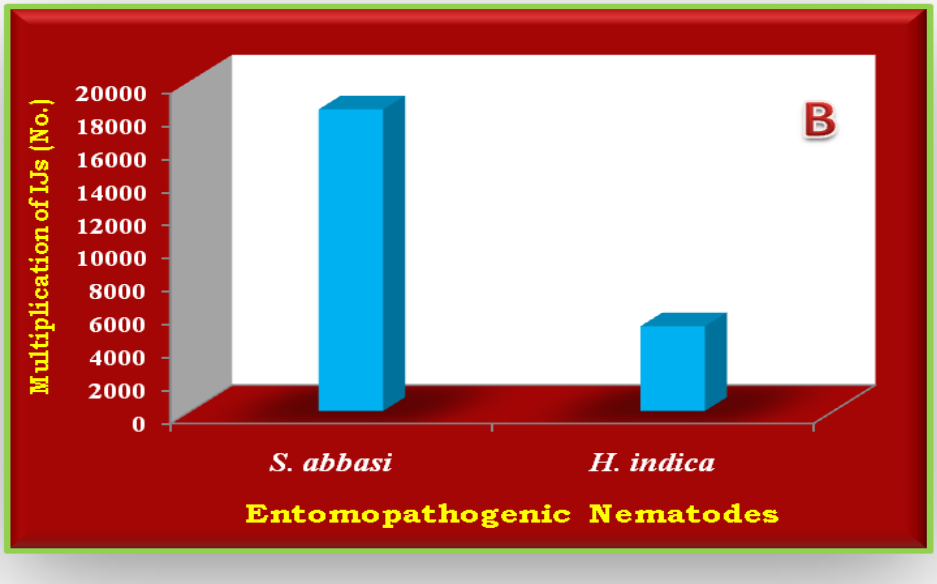

Fig. 10: Multiplication of EPNs on shoot borer larva

Effect of the temperature on infectivity and multiplication of EPNs

All the test eight species of EPNs were pathogenic against shoot borer larva at different temperatures. Mortality percentage of the test insect and multiplication of IJs varied from species to species of EPN.

***Effect of temperature on pathogenicity of EPNs against shoot borer larva*:** All the test EPN isolates were pathogenic against shoot borer larva at different temperatures but the rate of mortality was varied between species to species. Maximum mortality of insect was found at 30 ^{0}C followed by 28 ^{0}C, whereas the least mortality of insect was recorded at 20 and 35 ^{0}C (Fig. 11).

***Effect of temperature on multiplication of EPNs*:** Almost all the EPN isolates multiplied on the tested insect, however the level of multiplication varied within EPN isolates and temperatures regimes. Maximum number of IJs was yielded at 30 ^{0}C followed by 28 ^{0}C. Very poor multiplication was recorded at 20 and 35 ^{0}C. Among the tested EPN isolates, no multiplication of *Heterorhabditis* sp. (IISR 01), *S. ramanai*, *O. gingeri* was recorded at 20 ^{0}C, while *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02), *Oscheius* sp. (IISR 04) and *S. carpocapsae* at 35 ^{0}C (Fig. 12).

Mass production of EPNs

All EPNs isolates multiplied in the egg yolk and Wouts medium. Among these media, egg yolk was the best media for multiplication of IJs followed by Wouts media. Maximum number of IJs of *Oscheius* sp. (IISR 04) (37.5 IJs/flask ± 2.80, CV = 7.5 %) followed by *S. carpocapsae* (25.7 IJs/flask ± 5.71, CV = 22.6%), whereas, minimum number of *Oscheius* sp. (IISR 05) (13.2 IJs /flask ± 11.18, CV = 84.3%) were produced in egg yolk medium.

In case of Wouts media, out of eight tested EPNs, IISR-EPN 04 multiplied in highest number (23.5 IJs/flask ±5.25, CV = 22.4%) followed by *Steinernema* sp. (IISR 02) (23.1 IJs/flask ± 2.13, CV = 11.8%) whereas, lesser number of IJs produced of *Oscheius* sp. (IISR 08) (11.88 IJs/flask ± 6.88, CV = 57.9 %).

Very poor multiplications of tested EPNs was recorded in wheat flour medium. No multiplication was found in agar-agar and dog biscuit medium (Table 9).

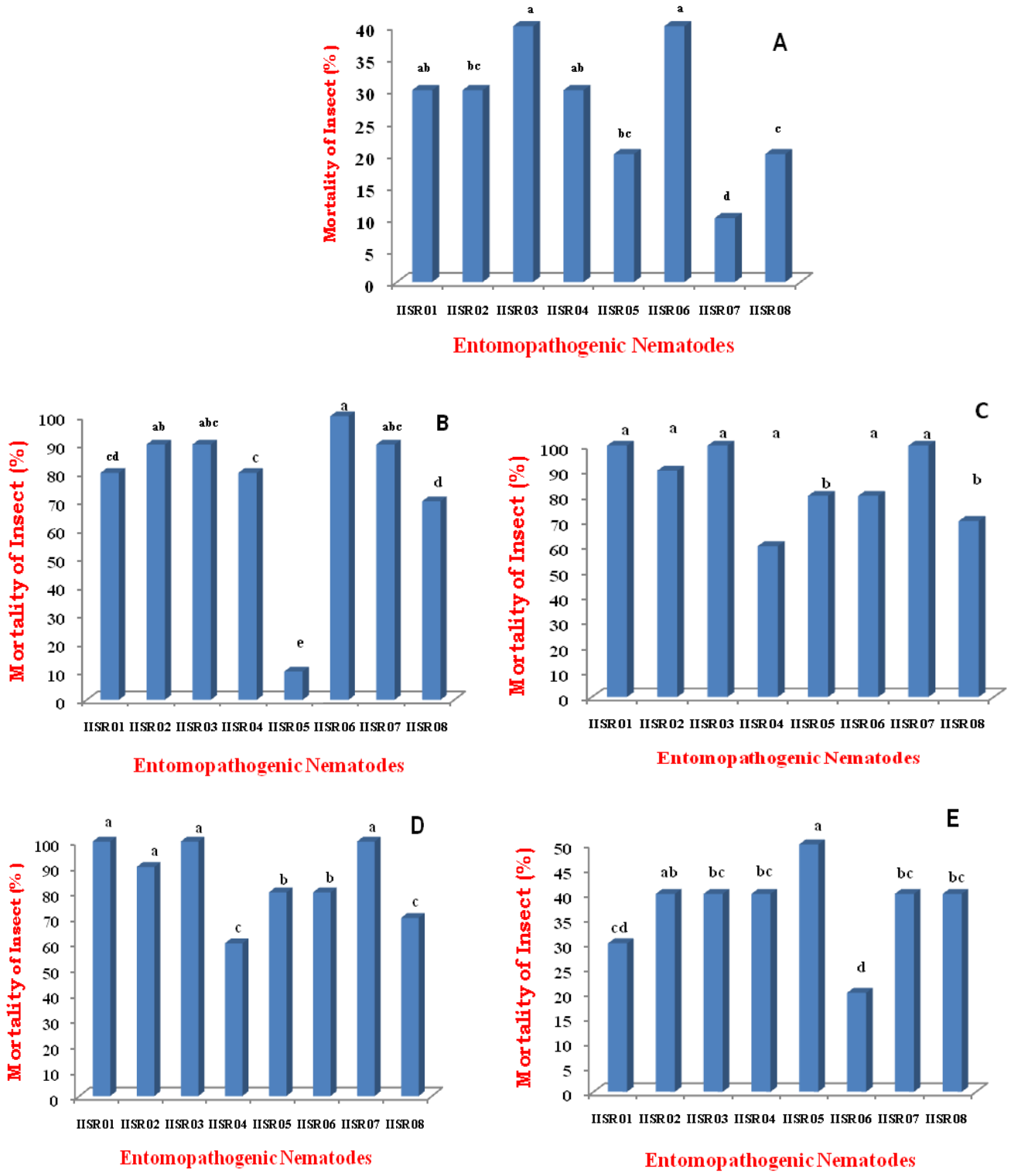

Fig. 11: Effect of temperature on efficacy of EPNs against shoot borer larva; **A**- 20 °C, **B** - 25 °C, **C**- 28 °C, **D** - 30 °C, **E** - 35 °C.

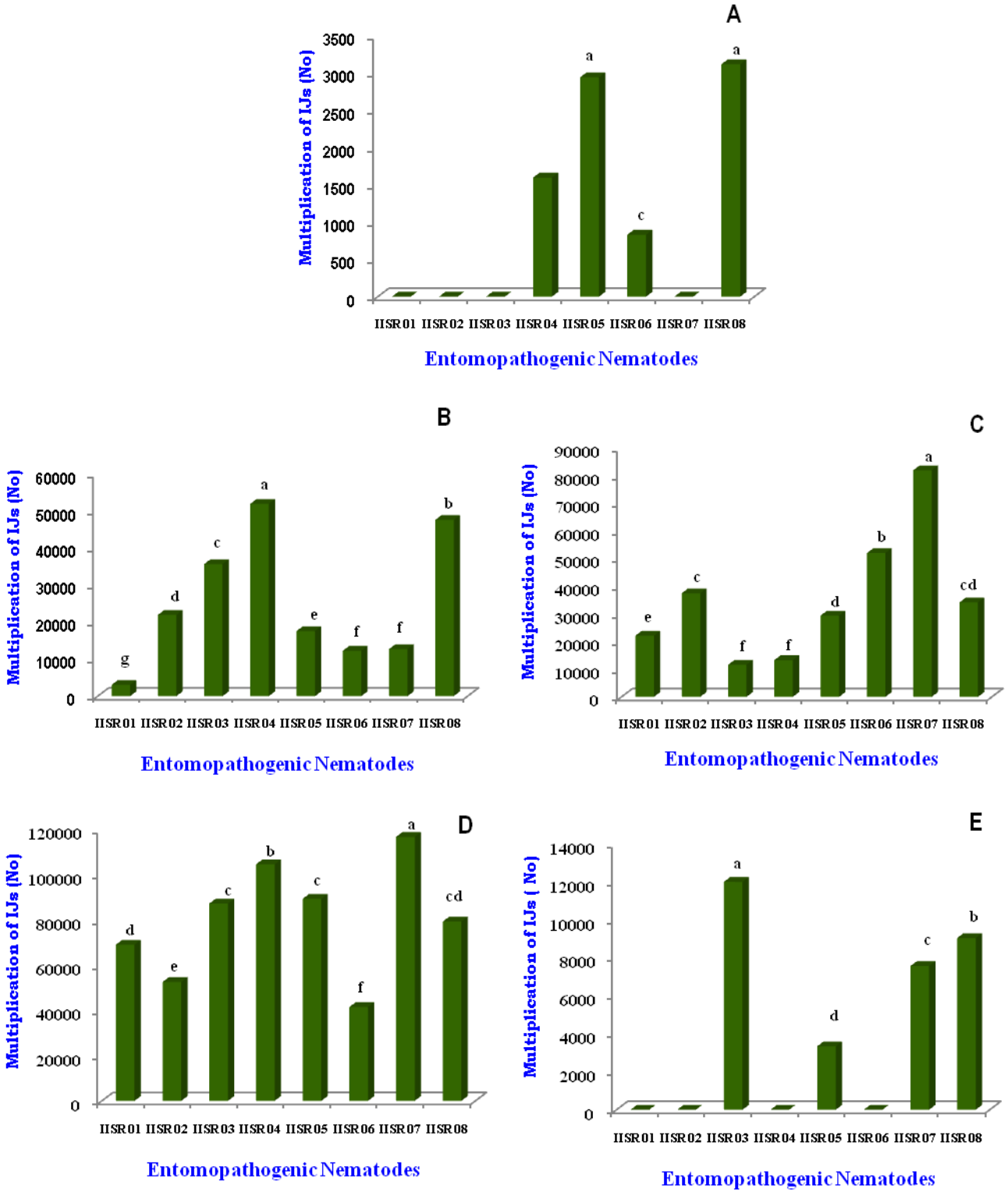

Fig. 12: Effect of temperature on multiplication of EPNs from shoot borer larva; **A-** 20 °C, **B** - 25 °C, **C-** 28 °C, **D** - 30 °C, **E** - 35 °C.

Table 9: Mass production of EPNs in different artificial media

EPNs	No. of IJs/flask (Lakh)*				
	WM	**EYM**	**WFM**	**DBM**	**AAM**
Heterorhabditis sp. (IISR 01)	15.32 ± 3.61 (12.83 - 21.63) 23.55	17.68 ± 5.21 (9.95 -22.12) 29.44	-	-	-
Steinernema sp. (IISR 02)	23.07 ± 2.13 (19.64 -26.45) 11.83	23.92 ± 3.03 (19.54 - 27.30) 12.67	-	-	-
S. ramanai	19.28 ± 2.92 (14.56 - 21.95) 15.15	27.89 ± 2.53 (23.84-30.43) 9.07	-	-	-
Oscheius sp. (IISR 04)	23.46 ± 5.25 (18.21- 30.32) 22.37	37.53 ± 2.80 (33.45-40.91) 7.46	0.312 ± 0.13 (0.13 - 0.49) 42.3	-	-
Oscheius sp. (IISR 05)	20.01 ± 7.59 (13.11-29.12) 37.89	13.25 ± 11.18 (0.63 - 23.46) 84.38	0.33 ± 0.40 (0 - 0.81) 118.68	-	-
S. carpocapsae	14.86 ± 3.52 (9.73 - 18.24) 23.68	25.71 ± 5.71 (18.98 - 32.19) 22.64	-	-	-
O. gingeri	21.56 ± 5.03 (16.08 - 29.56) 23.10	24.34 ± 7.78 (13.74 - 34.35) 31.92	0.40 ± 0.10 (0.28 - 0.52) 24.74	-	-
Oscheius sp. (IISR 08)	11.88 ± 6.88 (1.32 - 19.83) 57.86	25.21 ± 13.55 (8.13 - 37.29) 52.68	0.18 ± 0.13 (0.05 - 0.39) 70.49	-	-

* Data in the form of Mean ± SD (Range) and CV (%)

WM - Wouts; **EYM** - egg yolk; **WFM** - wheat flour; **DBM** - dog biscuit; **AAM** - agar agar medium

8222 Questions- Answered

1. Are any entomopathogenic nematodes available in spice agro ecosystems?
   Yes, entomopathogenic nematodes are available in spice agro ecosystems.
2. Can EPNs kill the shoot borer larva?
   Yes, EPNs are capable of killing shoot borer larvae based on laboratory bioassays.
3. How many promising isolates of EPNs have been identified against shoot borer larvae

   Four isolates of EPNs belonging to *Steinernema* sp (IISR 02), *Heterorhabditis* sp. (IISR 01) and *Oscheius* sp. (IISR 07 and 08) were promising against shoot borer larvae based on laboratory bioassays.
4. Can entomopathogenic nematodes be multiplied in artificial media?
   Yes, entomopathogenic nematodes can be multiplied in artificial media.
5. Are there new species of EPN in ginger rhizosphere?
   Yes, two new species *S. ramani* and *O. gingeri* collected from ginger rhizosphere have been described.

8223 Process/ Product/ Technology/ Developed:

- Identified two new species of entomopathogenic nematodes as *S. ramani* and *O. gingeri*, which can kill shoot borer larva and pupa within 72 h.
- Egg yolk and Wouts media were identified as promising media for the production of entomopathogenic nematodes.

8224 Practical Utility (not more than 150 words)

Four isolates viz., *Heterorhabditis* sp. (IISR 01), *Steinernema* sp. (IISR 02) and *Oscheius* sp. (IISR 07 and 08) were found promising against shoot borer larva, whereas *Oscheius* sp. (IISR 07) and *Steinernema* sp. (IISR 02) were found promising against shoot borer pupa. These EPNs can be evaluated in the green house and field for the management of shoot borer larva infesting ginger and turmeric. These EPNs can be multiplied large scale in egg yolk and Wouts media.

8225 Constraints, if any: Nil

823 Publications and Material Development

(One copy each to be supplied with this proforma.)

8231 Research papers

1. Pervez, R., Eapen, S.J., Devasahayam, S and Jacob, T.K. (2012). Efficacy of some entomopathogenic nematodes against insect pests of ginger and their multiplication. *Nematol medit*. 40 (1): 39-44.

2. Pervez, R., Eapen, S.J., Devasahayam, S. and Jacob, T.K. (2011). A new species of entomopathogenic nematode *Steinernema ramanai* (Rhabditida: Steinernematidae) from ginger *(Gingber officinae* Rosc.). *Zoo Taxa*, (communicated).

3. Pervez, R., Eapen, S.J., Devasahayam, S. and Jacob, T.K. (2011). A new species of entomopathogenic nematode *Oscheius gingeri* sp. n. (Nematoda: Rhabditidae) from ginger rhizosphere. *Archives of Phytopathology and Plant Protection*. (communicated).

8232 Popular articles

1. Pervez, R., Eapen, S. J. and Devasahayam, S. (2010). Keetnashak sutrakrimi: saflta ki kunzi. *Malabar Jyoti*, 37-38.
2. Pervez, R. and Eapen, S. J. (2011). Adrak mein sutrkirmiyoon ki samsya avam samadhan. *Spices India,(Hindi)*, 23 (11): 20-22.
3. Pervez, R. and Eapen, S. J. (2012). Masaloon mein sutrkirmiyoon ki samsya avam samadhan.*Masaloon ki Mehak*,38-40.
4. Pervez, R., Eapen, S. J. and Devasahayam, S. (2012). Adrak ko hani phochaney wale ketoon ka ketnashak sutkrimi duara Niyantaran. *Bhartiya Krishi Anusandhan Patrika* (accepted).

8233 Reports

Annual reports of IISR, Kozhikode 2009, 2010, 2011 and 2012

8234 Seminars, conferences and workshops (relevant to the project) in which the scientists have participated. (List abstracts forwarded)

Seminars

1. Pervez, R., Eapen, S.J., Devasahayam, S and Jacob, T.K. (2011). A new species of entomopathogenic nematode *Steinernema ramanai* (Rhabditida: Steinernematidae) from ginger (*Gingber officinae* Rosc.). In: 13th Indian Agricultural Scientists and Farmers Congress, Allahabad, 19-20 February, 2011.

2. Pervez, R., Eapen, S.J., Devasahayam, S and Jacob, T.K. (2011). Efficacy of entomopathogenic nematodes against insect pests of ginger and their multiplication. In: SYMSAC VI, Dharward (Karnataka), 8-10 December, 2011.
3. Pervez, R., Eapen, S.J., Devasahayam, S and Jacob, T.K. (2012). A new species of entomopathogenic nematode *Oscheius gingeri* sp. n. from ginger rhizosphere. In: 14th Indian Agricultural Scientists and Farmers Congress, Allahabad, 18-19 February, 2012.

8235 Awards:

- **Best Paper Presentation Award:** A new species of entomopathogenic nematode *Oscheius gingeri* sp. n. from ginger rhizosphere by Pervez, R., Eapen, S.J., Devasahayam, S and Jacob, T.K.. In: 14th Indian Agricultural Scientists and Farmers Congress, Allahabad from 18-19 Feb., 2012.
- **Best Poster Award**: A new species of entomopathogenic nematode *Steinernema ramanai* (Rhabditida: Steinernematidae) from ginger (*Gingber officinae* Rosc.) by Pervez, R., Eapen, S.J., Devasahayam, S and Jacob, T.K. In: 13th Indian Agricultural Scientists and Farmers Congress, Allahabad from 19-20 Feb., 2011.
- **Best Participant Award:** ICAR sponsored Summer School on Advances in Entomopathogenic Nematodes for Eco-Safe and Economic Pest Management at Rajasthan College of Agriculture, MPUA&T, Udaipur (Rajasthan) from 14 September to 4th October 2010.

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

1. Developed laboratory to maintain cultures and mass production of EPNs .
2. Developed laboratory to maintain cultures and mass production of *Galleria mellonella* on artificial diet.

Experimental Data Register Number: 114

Field Note Book Number: 114

Location: Nematology Section, Division of Crop Protection, IISR, Kozhikode

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

Evaluation of promising strains of entomopathogenic nematodes for reducing the damage of shoot borer infesting ginger and turmeric is to be taken up under green house and field conditions.

Part – IV: Project Expenditure

(Summary)

Year 2008 -12

630 Recurring Expenditure

6301 Salaries: (Designation with pay scale)

i) Scientific:

Principal Scientist (37000 - 67000)	12 man months	: 6, 65, 050
Sr. Scientist (37000 - 67000)	12 man months	: 5, 90, 730
Sr. Scientist (15600 - 39100)	18 man months	: 5, 52, 480

ii) Technical

Technical Assistant (5200 - 20200)	12 man months	: 1,63,710
Supporting (5200 - 20200)	9 man months	: 72,951

Sub Total	**: 20,44,921**

6302 Contingency

i) Chemical	: 90,000
ii) Glassware	: 65,000
iii) Special purchases	: Nil

Sub Total	**: 1,55,000**

6303	Travel	: Nil
6304	Miscellaneous (Other costs)	: 36,000
6305	Sub Total (Recurring)	**: 36,000**

631 Non Recurring Expenditure : Nil
(Equipments)

Grand Total (630 and 631)	**: 22,35,921**

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 and comments of the Head of the Division/Section:

Signature and Comments of the Director: