Forwarded 5/4/07

FAUNAL COMPOSITION AND DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES AND THEIR BIOEFFICACY AGAINST MAJOR INSECT PESTS IN RI-BHOI DISTRICT OF MEGHALAYA

ABSTRACT

BY

LALRAMLIANA

DEPARTMENT OF ZOOLOGY

SUBMITTED

in

FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

OF

NORTH-EASTERN HILL UNIVERSITY

SHILLONG – 793 022

samples were from the forest soils. *Steinernema* spp. were mostly isolated from sandy loam soils while *Heterorhabditis* sp., was isolated from red loamy soil.

Based on their morphometric measurements, and light/scanning electron microscopic studies the isolated nematodes were identified as, *Heterorhabditis indica, Steinernema thermophilum* and *Steinernema glaseri.* It is for the first time that their occurrence is reported herein from north-east region of India, Meghalaya in particular. A brief description is provided for all the species recorded.

For the study of seasonal population fluctuations of EPNs, two nematode positive sites, one for *Heterorhabditis* sp. and another for *Steinernema* sp. were selected in the study area. The seasonal prevalence was studied using indirect method and the presence of EPNs was evaluated using *Galleria* traps. *Heterorhabditis* sp. was detected in the soil samples throughout the study period, causing moderate to high mortality of the host insect. The prevalence of *Heterorhabditis* and *Steinernema* species was found to be positively correlated with soil temperature, soil moisture and rainfall. The prevalence of *Heterorhabditis* was recorded to be considerably high during March to November. The prevalence of *Steinernema* sp. started increasing form March onwards and reached a peak in May; thereafter it maintained almost a uniform trend till November. The prevalence declined abruptly to a very low level in the month of December.

With respect to ecological characterization of EPN species, effects of following parameters were taken into consideration: 1). temperatures on infectivity/production of IJs 2.) relative humidity on production of IJs 3). storage temperatures, population densities and duration on survival and pathogenicity of IJs 4). Time period and soil depth on attachment of IJs to a mobile host 5.) soil moisture levels on establishment of IJs in the host 6). IJs population densities on their infectivity/production 7). different pesticides on survival/pathogenicity of IJs.

Effect of temperatures on infectivity and reproduction of EPNs was determined by numbers of IJs established per *G. mellonella* and the numbers of IJs produced per insect host at different temperatures (10 – 35^{0}C). The optimum temperature (high establishment and production of IJs) of EPNs were recorded to be, *H. indica* from 20–30^{0}C, *S. thermophilum* from 25–35^{0}C and *S. glaseri* from 15-35^{0}C.

Effect of relative humidities on reproduction of IJs was determined by the first day of emergence of IJs and the number of IJs produced by the insect host exposed to 55 – 100% R.H. The optimum R.H range for all three species was found to be 80 – 100%.

To study the effect of storage temperature, population densities and duration, IJs of EPNs were exposed to four different temperatures (5, 10, 25 and 30^{0}C) and three population densities (100, 500 and 1000 IJs/ml) for a period of 120 days and their survival and pathogenicity were determined at different time intervals. Study revealed that 10^{0}C, 100

IJs/ml but not more than 60 days was the optimum condition for *H. indica* and *S. glaseri*, whereas 25°C, 100 IJs/ml but not more than 60 days was the best condition for *S. thermophilum*.

Foraging behavior of isolated EPNs was studied by two methods 1). attachment of IJs to a mobile host 2). capability of IJs to infect host at different soil depths. The attachment *H. indica* IJs to a mobile host was higher at all observation time (1, 5 and 10 minutes) as compared to *S. thermophilum* and *S. glaseri*. However, at different soil depths *S. glaseri* appeared as the most effective species as it showed the highest establishment of IJs in the host at the deepest soil depth of 10 cms.

To study the effect of soil moisture on infectivity of IJs, establishment of IJs in the insect host was observed at different soil moisture levels. It emerged that IJs of *S. thermophilum* could establish at 4% soil moisture level. At 5% moisture level IJs establishment was observed for all EPNs. Numbers of IJs established/host increased along the soil moistures reaching their peak at 16 – 18% soil moisture level and declined from 20% onwards, except for *S. glaseri* where the establishment rate increased till the highest soil moisture level studied.

The effect of IJs population densities on their infectivity and production were studied by exposing *G. mellonella* larvae to different concentrations of IJs. The infected dead larvae were observed for the establishment, first day of emergence and production of IJs of EPNs. It appeared that the number of IJs established per insect host significantly

increases at higher concentrations for all the EPNs. The first day of IJs emergence from the host cadavers was also affected by the population densities. Earlier emergences were observed at higher densities but no IJs were produced at establishment of IJs higher than 551.5 ± 41.92, 428.4 ± 74.73 and 321.4 ± 22.96 IJs per host for *H. indica, S. thermophilum* and *S. glaseri,* respectively. The highest numbers of IJs were produced at a concentration of 200 IJs/larva for both *H. indica* and *S. thermophilum.*

In order to ascertain the effect of different pesticides on survival and pathogenicity, IJs of EPNs were exposed to two (lower and higher) recommended field dose of carbaryl, nimbecidine, endosulfan, quinolphos, fenvalerate, mancozeb and carbofuran for a period of 72 hrs. It was observed that IJs of *H. indica* was found compatible with mancozeb and nimbecidine at both lower and higher concentrations. IJs of *S. thermophilum* were found compatible with carbaryl and nimbecidine at lower and higher concentrations, and to mancozeb at lower concentration, whereas IJs of *S. glaseri* were found compatible with nimbecidine, endosulfan, quinolphos, fenvalerate and mancozeb.

Bioefficacy of the isolated EPNs were tested against four major insect pests of the study area, namely larvae of colocassia corm borer, *Haplosonyx chalybaeus,* larvae of cabbage butterfly, *Pieris brassicae,* larvae and pupae of brinjal fruit and shoot borer, *Leucinodes orbonalis* and larvae and pupae of mustard saw fly, *Athalia lugens proxima.* Bioefficacy

was determined by percent insect larval/pupal mortality, and the total production of IJs per host to ensure their recycling potential.

The bio-efficacy of nematode species against *H. chalybaeus* larvae was studied by petridish assay. The larvae were exposed to 25, 50, 75, 100 and 200 concentrations of IJs for 120 hrs and host mortality was monitored at every 24 hrs intervals. 100% of larval mortality was observed for all EPNs at a concentration of 200 IJs/larva within 48 hours after inoculation (HAI) in case of *S. glaseri* and 72 HAI in case of both *H. indica* and *S. thermophilum.* Progeny production increased along with the IJs concentrations in EPN species. The maximum number of IJs produced was observed in case of *H. indica* (168.9 ± 2.67 x 10^3 IJs/larva at 200 IJs/larva) whereas the least infective juveniles yield was observed in case of *S. glaseri* (18.9 ± 0.57 x 10^3 IJs/larva at 100 IJs/larva).

Bioefficacy against larvae of *P. brassicae* was also studied using petridish assay with 10, 25, 50, 75 and 100 IJs concentration for a period of 120 hrs. The insect mortality was monitored at every 24 hrs intervals. Positive correlation was observed between the concentrations and the time of insect larval mortality. 100% of insect larval mortality was observed for all EPNs at a concentration of 200 IJs/larva within 48 HAI in case of *S. glaseri* and 72 HAI in case of both *H. indica* and *S. thermophilum.* The highest number of infective juveniles produced by *P. brassicae* was observed in case of *H. indica* (33.8 ± 2.46 x 10^3 IJs/larva at 100 IJs/larva).

In order to evaluate the bio-efficacy of nematode species against larvae and pupae of *L. orbonalis,* two assays were carried out *i.e.,* petridish assay for larvae and soil column assay for pupae. The larvae and pupae were also found to be susceptible to EPN species when exposed to different concentrations (10, 25, 50, 75 and 100 IJs/larva and 25, 50, 75, 100 and 200 IJs/pupa). In case of larvae, at the lowest concentration studied (10 IJs/larva), 100% mortality was observed only in case of *H. indica* within 120 HAI. At higher concentrations (100 IJs/larva) 100% mortality was observed for all the species within 48 HAI. The production of IJs by *L. orbonalis* showed the same trend as with other insect pests, where the maximum production of IJs was observed in case of *H. indica* (96.7 ± 2.11 x 10^3 IJs/larva at 100 IJs/larva).

Pupae of *L. orbonalis* were less susceptible to EPNs. At the highest concentration studied (200 IJs/larva), 100% mortality was observed within 96 HAI in case of *H. indica* (LC_{50} = 31.0), whereas *S. glaseri* caused only 53.4 ± 6.6% mortality even within 120 HAI (LC_{50} = 159.8). In case of *S. thermophilum,* an early mortality (20.00 ± 11.6%) was observed at 24 HAI which reached to 100% within 96 HAI (LC_{50} = 50.6). The pupa produced comparably less IJs than larva. Among the three species the production was observed highest in *H. indica,* yielding 12.2 ± 0.78 x 10^3 IJs/pupa at 200 IJs/pupa.

Bioefficacy against larvae and pupae of *A. proxima* were also studied by two assays (petridish assay for larvae and soil column assay for pupae)

at 10, 25, 50, 75 and 100 IJs/larva and 25, 50, 75, 100 and 200 IJs/pupa concentrations. In case of larvae, at the highest concentration (100 IJs/larva), 100% mortality was observed at 48 HAI for *H. indica* (LC_{50} = 30.6), whereas it was observed within 72 HAI (LC_{50} = 5.6) in *S. glaseri.* In case of *S. thermophilum,* mortality was first observed at 24 HAI which reached to 100% within 48 HAI (LC_{50} = 37.3). Of all the insect pest studied, the production of EPN IJs was comparably less in *A. proxima* larvae. The highest IJs produced were found to be only 29.3 ± 1.39 x 10^3 IJs/larva at 75 IJs/larva in case of *H. indica.*

The pupae were found less susceptible to EPNs as compared to larvae. No species could cause 100% pupal mortality. However, at the highest concentration of IJs (200 IJs/pupa), 93.4 ± 6.60% mortality was observed within 96 HAI in case of *H. indica* (LC_{50} = 81.7; LT_{50} = 56.1) and *S. glaseri* caused only 46.6 ± 6.60% mortality even within 120 HAI (LC_{50} = 197.3; LT_{50} = 112.3).

In conclusion, the present study shows that the EPN fauna in the Ri-Bhoi District of Meghalaya is comprised of three nematode species, i. e., *H. indica, S. thermophilum* and *S. glaseri.* On the basis of bioefficacy assays the study proves that all the EPN species have considerable potentials to be used as biocontrol agents against the tested insect pests.

FAUNAL COMPOSITION AND DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES AND THEIR BIOEFFICACY AGAINST MAJOR INSECT PESTS IN RI-BHOI DISTRICT OF MEGHALAYA

BY

LALRAMLIANA

DEPARTMENT OF ZOOLOGY

SUBMITTED

IN

FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

OF

NORTH-EASTERN HILL UNIVERSITY

SHILLONG – 793 022

DEDICATED TO MY LOVING PARENTS

(Zaikungi and Lalrinawma)

NORTH-EASTERN HILL UNIVERSITY

SHILLONG – 793 022, INDIA

5th April, 2007

DECLARATION

I, Lalramliana, hereby declare that the subject matter of this thesis is the record of the work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Zoology.

Lalramliana

(Candidate)

5/4/07

Prof. S.B. Prasad

(Head)

Dr. Arun Kumar Yadav

(Supervisor)

Contents

Acknowledgement

I would like to express my sincere appreciation and deep sense of gratitude to my supervisor, Dr. Arun Kumar Yadav, Reader, Department of Zoology, North-Eastern Hill University, Shillong, He, who guide me and lead me right from the beginning of my research journey. He is always there for me in every moment of my need for consultation, instruction, organizing plan of work, coordinating methodology and supervising overall design for the progress of work. For his kind acts of untiring help, constant guidance and continued encouragement throughout the study, I express my deepest and heartfelt gratitude to him.

I am also grateful to the present Head of the Department, Prof. SB Prasad, and former Heads of the department of Zoology, Prof. BBP Gupta and Prof. K Chatterjee (retd.) for providing me necessary laboratory facilities through out my research work. I thank all the faculty members of the department as well as non teaching staff for their suggestions and positive criticisms to attain this stage of my research.

My indebted gratefulness goes to Prof. V. Tandon, Dean, School of Life Sciences, NEHU, Dr. AN Shylesha, Senior Scientist, Indian Council of Agriculture Research, Umiam, Meghalaya and Dr. B. Kharbuli, Professor, Department of Zoology, NEHU for their untiring help, suggestions and continued encouragement throughout the study.

I expressed my sense of indebtness to my lab mates Among, Vareishang, and Mrs. Pde for their kind help in my research work and in times of need. My thanks are due to my close friends – Arun Rabha, Bidhya, Dindin, Vena, Zautea, Siam, Khaipu, HT, KC, PC, Saipari, Tawki, Thlatea, Kuheli, Shritapa, Zaiba, Pramod, Larisha, Sama,

need. Since my work needs a lot of field survey, helping hands received from the two best drivers, Bah Edwin and Pradhan (Sanker) are worth a mention here. Photographic arrangement done by Guesstar; continous help from Kong Dohling, Bah Deng, Vanlal are thankfully acknowledged.

I express my unbound respect to my parents for their continuous encouragement, loving care, silent muse of inspiration and financial support right from my infancy to this stage. Behind every successful man there is a woman, I express my heart felt thanks to my wife and my closest friend Zohmingthangi (Nute-i), thank you Nute for all the things you've done to me. I express my love to my sister Mami; my brothers Mama and Mapuia; my kids and all my close relatives, as you are my shield in the time of mental and physical storm.

The award of JRF (NET) by joint CSIR-UGC, New Delhi, and thereafter a research fellowship awarded by UGC, New Delhi is thankfully acknowledged.

Above all I thank God the Almighty for giving me strength to complete this work.

DATE: 05.04.2007
PLACE: **Shillong**

(LALRAMLIANA)
Department of Zoology,
North-Eastern Hill University,
Shillong – 793 022

Preface

Growing public concern over the consequences of use of chemical pesticides, particularly ground water contamination, residues on food, resistance development and wild life kills have fueled an intense search for safer alternatives. Biological control of insect pests is an ideal alternative, is economical and does not have any impact on non-target organisms. Biological control offers a tremendous opportunity to supply agriculture with effective tools for the development of production techniques, which minimize impacts on human health and the environment.

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* have been found in many diverse climates throughout the world. They have an enormous potential for insect pest control. These nematodes are able to invade and, in most cases, to kill a large number of insect pests. In the soil, a free-living stage known as the infective juvenile (IJ) penetrates insect hosts through natural body openings such as mouth and spiracles. Once inside the host, host specific, symbiotic bacteria (*Xenorhabdus* spp. for *Steinernema* spp. and *Photorhabdus* spp. for *Heterorhabditis*) are released from the IJ gut into the insect body cavity, thereby, precipitating a bacterial septicemia. The combination of nematode/bacterial virulence factors kills the host within 24-48 hours. Interest in EPNs as biological control agents of insect pests has increased

rapidly because they are extraordinarily lethal and kill the target insects within short period of time and are also safe to non-target organisms.

EPNs can provide effective biological control of some important soil insect pests and pests that occur in cryptic habitats. Accordingly there is an intense interest to isolate these nematodes from different regions of the world that have climatically adapted and have the potential for biological control of pests in that area. The species and isolates of EPNs exhibit considerable variations in term of host range, reproduction, infectivity and other ecological traits. In order to explore their potentials as biocontrol agents, locally adapted species or isolates from native habitats need to be identified and their unique characteristics documented.

Keeping in mind the aforesaid account, it was felt necessary to carry out a study on faunal survey, distribution of entomopathogenic nematodes and their bioefficacy against major insect pests in the Ri-bhoi District of Meghalaya. The focal aspects of the study include:

1. Establishing the faunal composition and distribution of EPNs in the Ri-Bhoi district, Meghalaya.
2. Studying the population fluctuations of EPNs with respect to climatic factors.
3. Characterizing locally isolated EPN species with respect to ecological parameters.
4. Evaluating the bioefficacy of EPN species against four major insect pests of the area.

Abbreviations

@	-	at the rate of
cm	-	centimeter
conc.	-	concentration
dia.	-	diameter
EPN	-	Entomopathogenic Nematode
et al.	-	et alii/alia (Latin: and others)
Fig.	-	Figure
gm	-	gram
HAI	-	Hours after inoculation
hrs	-	hours
IJs	-	Infective Juveniles
LC_{50}	-	Lethal Median Concentration
LT_{50}	-	Lethal Median Time
mm	-	millimeter
min.	-	minute
µm	-	micrometer
SEM	-	Standard Error of Mean
SPSS	-	Statistical Programme for Social Science
w/w	-	weight by weight

Abbreviations used in morphometric measurements

BL	-	Body Length
BW	-	Body Width
ES	-	*Oesophagous length from anterior end*
EP	-	Excretory pore position from anterior end
NR	-	Nerve ring position from anterior end
ABW	-	Anal body width

TL	-	Tail length
V%	-	Percentage of vulva position to body length
SPL	-	Spicule Length
SW	-	Spicule Length/Anal Body Width
GL	-	Gubernaculum Length
A	-	Body Length/Body Width
B	-	Body Length /Oesophagous Length
C	-	Body Length /Tail Length
D	-	Excretory pore position/Oesophagous Length
E	-	Excretory pore position /Tail Length
F	-	Body Width/Tail Length

CHAPTER 1

Prevalence and Distribution of Entomopathogenic Nematodes

Introduction

Entomopathogenic nematode (EPN) species of the genera *Steinernema* Travassos, 1927, *Heterorhabditis* Poinar, 1976 and *Neosteinernema* Nguyen and Smart, 1994 (Rhabditida: Nematoda) have attracted the attention of entomologists (and of nematologists) for a number of years for use in controlling economically important insect pests. With increasing restrictions on the use of chemicals and the mounting problem of resistance, such nematodes are valuable addition to the range of biological control agents available for insect pest management since they possess many of the attributes of effective biological control agents. Interest in entomopathogenic nematodes (EPNs) as biological control agents of insect pests has increased rapidly because they:

1. are extraordinarily lethal and kill the target insects within short period of time (24 - 48 hours);
2. can actively find their hosts and can also recycle in the soil environment (Kaya and Gaugler, 1993);
3. are environmentally safe (Akhurst, 1990);
4. have broad host range.
5. are safe to non-target organisms (Bathon, 1996);
6. can be made available commercially as formulations.

Steinernematid and heterorhabditid nematodes are obligate pathogens that infect a wide range of insects, and are characterized by their association with symbiotic bacteria carried in their digestive tract; *Xenorhabdus* spp. in steinernematids, and *Photorhabdus* spp. in heterorhabditids (Boemare *et al.*, 1996). Figure 1.1 illustrates the life cycles of steinernematid and heterorhabditid nematodes, they have a similar life cycle, except for a few differences. Their entry into the host is usually through natural openings such as the mouth, anus or spiracles (Triggiani and Poinar, 1976; Mrácek *et al.*, 1988). Infective juveniles (IJs) of heterorhabditids, unlike steinernematids, possess a 'tooth' that enables them to penetrate through soft cuticle (Bedding and Molyneux, 1982), peritrophic membranes and the midgut wall (Poinar and Georgis, 1990; Forschler and Gardner, 1991). The bacteria released by steinernematid and heterorhabditid nematodes rapidly multiply and the kills the host by septicemia within 24 - 48 hours (Poinar, 1983). The nematodes feed on the bacteria and nutrients made available because of bacterial digestion. One of the major differences between steinernematids and heterorhabditids is their development subsequent to the infective stage (Poinar, 1990). In *Steinernema spp.*, the IJs develop into amphimictic females or males, whereas in *Heterorhabditis* spp., each IJ develops into a hermaphroditic female and never an amphimictic female or male. However, the second generation (progeny of the initial females formed from the IJs) consists of amphimictic females and males in both genera. The nematodes may

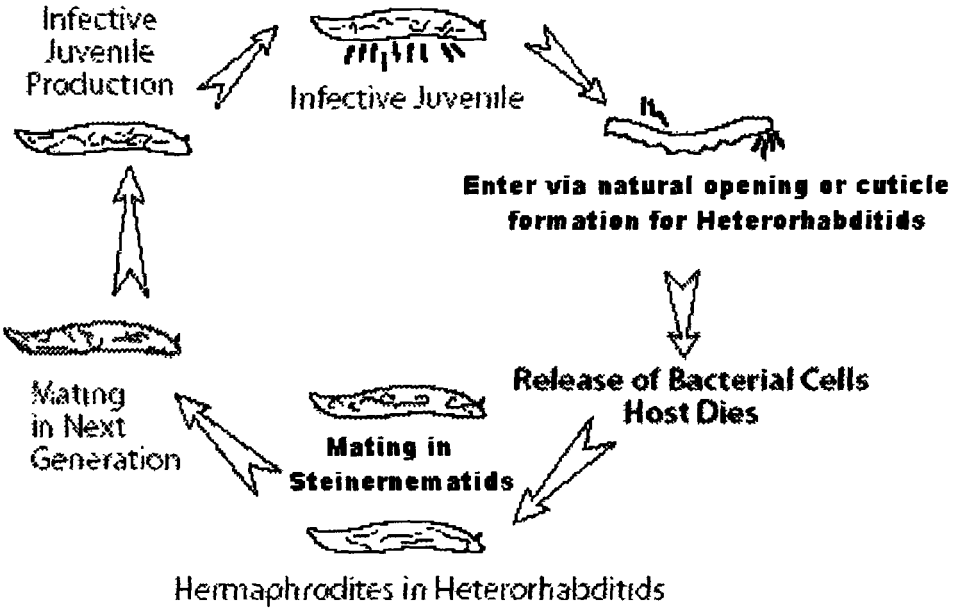


Fig. 1.1. General life cycle of entomopathogenic nematodes.

undergo one or two generations depending on the level of parasitism and the resources provided by the host. Depletion of nutrients and overcrowding induce the formation of IJs, which leave the cadaver in search of a new host (Kaya and Burlando, 1989; Popiel *et al.*, 1989).

Taxonomy

Like many other taxonomic classifications of organisms, that of entomopathogenic nematodes has also gone through a number of major changes. Steiner (1923) described the first steinernematid as *Aplectana kraussei*, which was later placed in the genus *Steinernema* by Travassos (1927). In 1929, Glaser and Fox (1930) discovered a nematode parasitizing the Japanese beetle, *Popillia japonica*, and upon examination, Steiner (1929) created the genus *Neoaplectana* and named the species as *N. glaseri*. Both genera were accepted and placed in the superfamily Steinernematidae by Filipjev (1934), later redefined as a family. Detailed examination of the two genera resulted in *Neoaplectana* being synonymised under *Steinernema* (Poinar, 1990). So far there are 47 species of *Steinernema* described from various regions of the world (Table 1.1), but a perusal of literature reveals that many more new isolates of *Steinernema* sp. have been collected that are still awaiting formal identification. The genus *Neosteinernema* was created in the family Steinernematidae, following the discovery of *Neosteinernema longicurvicauda* isolated from the termite, *Reticulitermes flavipes* (Nguyen and Smart, 1994).

Table 1.1: Taxonomic summary of the family Steinernematidae Chitwood and Chitwood, 1937

Taxa	Biogeography[a]
Type genus:	
Steinernema Travassos, 1927	
Type species:	
Steinernema kraussei (Steiner, 1923) Travassos, 1927	Europe (Germany), N. America
Other species:	
S. abbasi Elawad, Ahmad and Reid, 1997	Asia (Oman)
S. aciari Qiu, Yan, Zhou, Nguyen and Pang, 2005	Asia (China)
S. akhursti Qiu, Hu, Zhou, Pang and Nguyen, 2005	Asia (China)
S. apuliae Triggiani, Mracek and Reid, 2004	Europe (Italy)
S. affine (Bovien,1937) Wouts, Mracek, Gerdin and Bedding, 1982	Europe (Denmark)
S. anatoliense Hazir, Stock and Keskin, 2003	Asia (Turkey)
S. arenarium (Artyukhovsky, 1967) Wouts, Mracek, Gerdin and Bedding, 1982	Asia (Central Russia)
S. asiaticum Anis, Shahina, Reid and Rowe, 2002	Asia (Pakistan)
S. beddingi Qiu, Hu, Zhou, Pang and Nguyen, 2005	Asia (China)
S. bicornutum Tallosi, Peters and	Europe (Yugoslavia)

Continued....

Ehlers, 1995	
S. carpocapsae (Weiser, 1955) Wouts, Mracek, Gerdin and Bedding, 1982	Asia, Europe (Czechoslovakia), N. America, S. America
S. caudatum Xu, Wang and Li, 1991	Asia (China)
S. ceratophorum Jain, Reid and Hunt, 1997	Asia (China)
S. cubanum Mracek, Hernandez and Boemare, 1994	Central America (Cuba)
S. diaprepesi Nguyen and Duncan, 2002	North America (USA)
S. feltiae (Filipjev, 1934) Wouts, Mracek, Gerdin and Bedding, 1982	Europe (Denmark), N. America, S. America
S. glaseri (Steiner 1929) Wouts, Mracek, Gerdin and Bedding, 1982	Asia, Europe, N. America (USA), S. America
S. guangdongense Qiu, Fang, Zhou, Pang and Nguyen, 2004	Asia (China)
S. hermaphroditum Stock, Griffin and Chaerani, 2004.	Asia (Indonesia)
S. intermedium (Poinar, 1985) Mamiya, 1988	N. America (USA), Europe
S. jollieti Spiridonov, Krasomil-Osterfeld and Moens, 2004	N. America (USA)
S. karii Waturu, Hunt and Reid, 1997	Africa (Kenya)
S. kushidai Mamiya, 1988	Asia (Japan)
S. litorale Yoshida, 2004	Asia (Japan)
S. loci Phan, Nguyen and Moens, 2001	Asia (Vietnam)
S. longicaudam Shen, 1992	Asia (China), N. America
S. monticolum Stock, Choo and Kaya, 1997	Asia (Korea)

Continued.....

S. neocurtillae Nguyen and Smart, 1992	N. America (USA)
S. oregonense Liu and Berry, 1996	N. America (USA)
S. pakistanense Shahina, Anis, Reid, Rowe and Maqbool, 2001	Asia (Pakistan)
S. puertoricense Roman and Figueroa, 1994	Central America (Puerto Rico)
S. rarum (Doucet, 1986) Mamiya, 1988	S. America (Argentina), N America (USA)
S. riobrave Cabanillas, Poinar and Roulston, 1994	N. America (USA)
S. ritteri Doucet and Doucet, 1990	S. America (Argentina)
S. robustispiculum Phan, Subbotin, Waeyenberge and Moens, 2005.	Asia (Vietnam)
S. sangi Phan, Nguyen and Moens, 2001	Asia (Vietnam)
S. scapterisci Nguyen and Smart, 1990	S. America (Uruguay)
S. scarabaei Stock and Koppenhofer, 2003	N. America (USA)
S. serratum Liu, 1992[b]	Asia (China)
S. siamkayai Stock, Somsook and Reid, 1998	Asia (Thailand)
S. silvaticum Sturhan, Spiridonov and Mracek, 2005	Europe (Germany)
S. tami Luc, Nguyen, Reid and Spiridonov, 2000	Asia (Vietnam)
S. thanhi Phan, Nguyen and Moens, 2001	Asia (Vietnam)
S. thermophilum Ganguly and Singh, 2000	Asia (India)

Continued.....

S. websteri Cutler and Stock, 2003	Asia (China)
S. weiseri Mracek, Sturhan and Reid, 2003.	Europe (Czech Republic)
S. yirgalemense Nguyen, Tesfamariam, Gozel, Gaugler and Adams, 2004.	Africa (Ethiopia)
Genus: *Neosteinernema* Nguyen and Smart, 1994	
Type and only species:	
Neosteinernema longicurvicauda Nguyen and Smart, 1994	N. America (USA)

[a] Country of original isolation in parentheses
[b]Species*inquirenda*

The family Heterorhabditidae was created by Poinar (1976) containing the species *H. bacteriophora*. As with the steinernematids, the species in the Heterorhabditidae have also undergone a number of taxonomic changes (Mason *et al.*, 1996). It is important to note that many of these species are further classified by strain, since isolates of the same species collected from different geographical regions vary significantly in their behavioural and physiological adaptations. Currently, there are 12 species of *Heterorhabditis* described so far from various regions of world (Table 1. 2).

Occurrence and Distribution of Entomopathogenic Nematodes:

Entomopathogenic nematode (EPN) species of the genera *Steinernema* Travassos, 1927, *Heterorhabditis* Poinar, 1976 and *Neosteinernema* Nguyen and Smart, 1994 (Rhabditida: Nematoda) have been reported to occur in the tropical, subtropical and temperate countries (Kaya, 1990). The only continent where they have not been found to occur is Antarctica (Griffin *et al.*, 1990). Numerous surveys for EPNs have been conducted throughout the world by using baiting technique (Bedding and Akhurst, 1975). The isolation of EPNs from all inhabited countries has been well documented by Kaya (1990). There is an intense interest to isolate these nematodes from different regions of the world, which are climatically adapted and have the potential for biological control of pests in that area. Many countries are concerned about the introduction of exotic species of EPNs, because of their

Table 1.2 : Taxonomic summary of the family Heterorhabditae Poinar, 1976

Taxa	Biogeography[a]
Type and only genus:	
Heterorhabditis Poinar, 1976	
Syn. *Chromonema* Khan, Brook and Hirschmann, 1976	
Type species:	
Heterorhabditis bacteriophora Poinar, 1976	Africa, Asia, Australia, Central America, Europe, N. America (USA), S. America
Syn. *Chromonema heliothidis* Khan, Brook and Hirschmann, 1976	
H. heliothidis (Khan, Brook and Hirschmann, 1976) Poinar, Thomas and Hess, 1977	
H. argentinensis Stock 1993[b]	
Other species:	
H. baujardi Phan, Subbotin, Nguyen and Moens, 2003	Asia (Vietnam)
H. brevicaudis Liu, 1994	Asia (China)
H. downesi Stock, Burnell and Griffin, 2002	Europe (Ireland)
H. floridensis Nguyen, Gozel, Koppenhofer and Adams, 2006	N. America (USA)
H. indica Poinar, Karunakar and David, 1992	Asia (India), Central America
Syn. *H. hawaiiensis* Gardner, Stock and Kaya, 1994[b]	N. America
H. marelata Liu and Berry, 1996	N. America (USA)
Syn *H. hepialius* Stock, Strong and	

Continued.....

Gardner, 1996	
H. megidis Poinar, Jackson and Klein, 1987	N. America (USA)
H. mexicana Nguyen, Shapiro-Ilan, Stuart, James, McCoy and Adams, 2004	Central America (Mexico)
H. poinari Kakulia and Mikaia, 1997[c]	Europe (Georgia)
H. taysearae Shamseldean, Abou El-Sooud, Abd-Elgawad and Saleh, 1996	Asia (Egypt)
H. zealandica Poinar, 1990	Australia (New Zealand)

[a] Country of original isolation in parentheses
[b] As proposed by Stock
[c]Species *inquirenda*

negative impacts on non-target organisms. Hence, surveys have been conducted in many parts of the world demonstrating their wide spread occurrence and providing an indication of which species are indigenous for a given area.

Natural distribution:

In Europe (Czechoslovakia), Steiner (1923) described the first steinernematid as *Aplectana kraussei*, which was later placed in the genus *Steinernema* by Travassos (1927). Again from Czechoslovakia, Weiser (1955) isolated a new steinernematid and named it as *Steinernema carpocapsae.* The other reports of EPNs from Czechoslovakia include those of Mracek *et al.* (1982), Miduturi *et al.* (1997), Mracek *et al.* (1999), who reported *S. feltiae, S. bicornutum, Heterorhabditis megidis, S. affine, S. intermedium* and *S. kraussei* from this country. Recently, Mracek and Becvar (2000) also reported *S. kraussei, S. feltiae, S. affine, S. intermedium, S. bicornutum* and two undescribed species of *Steinernema* from Czechoslovakia The two undescribed species were later described as a new species, *S. weiseri* by Mracek *et al.* (2003). They mentioned that *S. weiseri* is also widely distributed in Germany and Slovakia.

While surveying Belgium soils, Miduturi *et al.* (1997) isolated three species of EPNs namely, *H. megidis, S. affine* and *S. feltiae.* Recently, Ansari *et al.* (2005) reported for the first time occurrence of *S. glaseri,* in Belgium from a natural host, *Hoplia philanthus* (Coleoptera: Scarabaeidae). In Denmark, Filipjev (1934) initiated the studies on

EPNs by isolating a new species, *Steinernema feltiae*. Bovien (1937) in addition of reporting *S. feltiae* also described another new species, *S. affine* from Denmark. Poinar (1985; 1986) reported *S. feltiae* and *S. carpocapsae* from France. The same worker also reported *S. feltiae* from Finland (Poinar, 1990). In Germany there are a number of studies on EPNs documenting the occurrence of *S. affine* (Poinar, 1990), *S. kraussei* (Mracek, 1994), *H. bacteriophora* (Glare *et al.*, 1993; Sturhan, 1996), *H. megidis* and *S. feltiae* (Sturhan, 1996.). Recently, Sturhan et al. (2005) described *S. silvaticum* from Berlin (Germany).

Surveying the soils of Netherlands, Hominick *et al.* (1995) isolated *H. megidis, S. affine, S. feltiae* and *S. kraussei*. Mracek and Jenser (1988), reported the occurrence of *Heterorhabditis* sp., *S. carpocapsae* and *S. feltiae* from Hungary. Similarly, *S. affine* and *H. megidis* have also been reported to occur in Ireland (Griffin *et al.*, 1991; Joyce *et al.*, 1994). Stock *et al.* (2002) described a new species of EPN from Ireland, *H. downesi*. From Italy, Tarasco and Triggiani (1996) reported *S. affine* and *H. bacteriophora*. Poinar and Veramchuk (1970), while studying the distribution of *S. carpocapsae,* reported their presence in Leningrad, USSR. In Norway, studies on EPNs have been made by Haukeland (1993) who recorded occurrence of *S. feltiae*. No further studies have been reported other than this study. From Poland, only two species of EPNs have been reported so far *viz, S. carpocapsae* (Stanuszek, 1974) and *S. feltiae* (Sandner and Bednarek, 1987). Boag *et al.* (1992) and Gwyn and Richardson (1996) reported the occurrence of EPNs in Scotland. Tallosi *et al.* (1995) described a new species from

EPNs in Scotland. Tallosi *et al.* (1995) described a new species from Serbia and named it as *Steinernema bicornutum.* From Slovak Republic, Sturhan and Liskova (1999) reported *S. affine, S. carpocapsae, S. feltiae, S. intermedium* and *S. kraussei.* Doucet and Gabbarra (1994) conducted surveys in Spain and reported the presence of *H. bacteriophora* and *S. feltiae.* In Sweden, Burmann *et al.* (1986) and Poinar (1986) reported the presence of EPNs. Steiner (1994) first reported the occurrence of EPNs in Switzerland soils. Steiner (1996) again conducted another survey and altogether *S. affine, S. feltiae, S. intermedium* and *S. kraussei* were reported from Switzerland. Ozer *et al.* (1995) for the first time reported *S. feltiae* from Turkey. Hazir *et al.* (2003a) while studying the distribution of EPNs in Turkey, isolated *H. bacteriophora, S. feltiae, S. affine* and one new *Steinernema* sp., which they later described as *Steinernema anatoliense* (Hazir *et al.*, 2003b). Artyukhovsky *et al.* (1997) described a new species, *Steinernema arenarium* from a central part of Russia.

In other parts of Europe, such as Yugoslavia and United Kingdom surveys to document the occurrence of EPNs have also been carried out by Tallosi *et al.* (1993), Hominick and Briscoe (1990), Hominick *et al.* (1995), Gwynn and Richardson (1996) and Rosa *et al.* (2000).

In both the continents of America, Glaser and Fox (1930) initiated the studies on EPNs by discovering a nematode parasitizing the Japanese beetle, *Popillia japonica,* in New Jersey, USA. This nematode is what now called as *Steinernema glaseri.* From time to time different workers continued making surveys of EPNs in various parts of USA and

isolated several species of nematodes. The new species described from different parts of USA include, *S. intermedium* (Poinar, 1985) from South Carolina, *H. megidis* (Poinar *et al.*, 1987) from Ohio, *S. neocurtillae* (Nguyen and Smart, 1992) from Florida, *H. hawaiiensis* (Gardner *et al.*, 1994) from Hawaiian Island, *S. riobrave* (Cabanillas *et al.*, 1994) from Texas, *S. aregonense* (Liu and Berry, 1996a), and *H. maurelatus* (Liu and Berry, 1996b). Other than these, new species like *S. diaprepesi* (Nguyen and Duncan, 2002), *S. scarabaei* (Stock and Koppenhofer, 2003) and *S. jollieti* (Spiridonov *et al.*, 2004) were also described from USA. Recently, Nguyen *et al.* (2006a) described *H. floridensis* from Florida, USA.

In Argentina, Poinar and Veramchuk (1970) first reported the presence of *S. carpocapsae*. Later, Stock (1993) described a new species of heterorhabditid, *H. argentinensis* from this country. Doucet (1986; 1990) described another two new species of EPNs namely, *S. rarum* and *S. ritteri* from Argentina. Later, Stock (1995) reported the presence and diversity of EPNs in this country. Caicedo and Bellotti (1996) reported the presence of *H. bacteriophora* from Columbia. Poinar (1986) reported the presence of *S. carpocapsae* in Canada, while Mracek and Webster (1993) reported the presence of *H. megidis and* other *Steinernema* spp. from Western Canada. Mracek *et al.* (1994) conducted survey and described a new species, *S. cubanum* from Cuba. Constant *et al.* (1998) while surveying Guadaloupe Islands reported the presence of EPNs, namely, *H. bacteriophora, H. indica* and *Steinernema* sp. Roman and Fegueroa (1994) described *S. puertoricense* from Puerto Rico. In Mexico

Nguyen *et al.* (2004b) described *H. mexicana.* In Uruguay, Nguyen and Smart (1990) discovered a new steinernematid from mole cricket and named it as *S. scapterisci.*

In other parts of the world like Australia, surveys were conducted by Akhurst (1980), who reported the presence of *S. carpocapsae* in Australian soils. Similarly, Poinar (1986) also reported the presence of *S. carpocapsae* along with *S. feltiae* in Australia. Akhurst and Bedding (1986) reported the presence of steinernematid and heterorhabditid, and their frequency of occurrence was recorded to be 8.2%. Akhurst (1987) while surveying some parts of Australia and New Zealand reported the presence of *H. bacteriophora* and *H. zealandica* in these countries. Wouts (1980) and Curan (1989) in their studies reported *S. feltiae* and *H. zealandica* from New Zealand.

In Africa, Waturu *et al.* (1997) reported *S. karii* from Kenya. Nguyen *et al.* (2004a) reported *S. yirgalamense* from Ethiopia and more recently, Nguyen *et al.* (2006b) reported *S. khoisanae* from South Africa.

Surveys for EPNs have also been undertaken in Asia for documentation of EPN species in different countries. Mamiya (1988) isolated a new species from Japan and described it as *S. kushidai.* Yoshida *et al.* (1998) while conducting a survey in Japan reported both steinernematid and heterorhabditid, having their frequency of occurrence as 8.15 and 2.0%, respectively. Later, Yoshida (2004) isolated *S. litorale* from sandy soils of Honshu Island, Japan. In China, Li and Wang (1989) reported both *S. glaseri* and *H. bacteriophora,* Similarly, Xu *et al.* (1991) described a new species, *S. caudatum* from

China. Liu (1992; 1994) also described *S. cerratum* and *H. brevicaudis* from China. From the same country, Jain *et al.* (1997) described another new species, *S. ceratophorum,* and Shen (1992) described *S. longicaudam.* Later, Cutler and Stock (2003) reported another new steinernematid from China which was named as *S. websteri.* Qiu *et al.* (2005 a, b, c) described three new steinernematids *viz., S. aciari, S. akhursti* and *S. beddingi,* all from Yunan Province, China.

Shamseldean and Abd-Elgawad (1994) conducted survey for EPNs in Egypt and isolated *Heterorhabditis* sp. in 9.5% of soil samples. From Egypt, Shamseldean *et al.* (1996) described a new species, *H. taysearae.* Amarasinghe *et al.* (1994) reported the presence of *H. indica* from Sri Lanka and the same species along with *Steinernema* spp. was also reported by Mason *et al.* (1996) from Malaysia. From Pakistan, two new EPN species described include, *S. pakistanense* (Shahina *et al.*, 2001) and *S. asiaticum* (Anis *et al.*, 2002). Choo *et al.* (1995) isolated *H. bacteriophora* and *Steinernema* sp. from Korea. Later Lee *et al.* (1996) reported the presence of *Steinernema* sp. and *Heterorhabditis* spp. from S. Korea. A new species, *S. monticolum* has also been reported from Korea by Stock *et al.* (1997).

From the Sultanate of Oman, Elawad *et al.* (1997) described one new species, *S. abbasi,* the same species was also isolated from Taiwan (Liao *et al.*, 2001). Stock *et al.* (1998) also described a new EPN species from Thailand which was named as *S. siamkayai.* Tangchitsomkid and Sonitrat (1998) conducted survey in Thailand and reported the frequency of occurrence of EPNs to be 12.5%. Griffin *et al.* (2000)

reported the presence of *H. indica* in Indonesia, and later Iraki *et al.* (2000) reported the same species from West Bank, Palestina. Luc *et al.* (2000) described a new species, *S. tami* from Vietnam. Other species like, *S. sangi* (Phan *et al.*, 2001a), *S. loci* and *S. thanhi* (Phan *et al.*, 2001b), *H. baujardi* (Phan *et al.*, 2003) and *S. robustispiculum* (Phan *et al.*, 2005) have also been described from Vietnam.

The work on EPNs in India was first started in 1966 by Rao and Manjunath, on biocontrol potentials of DD-1136 strain of an exotic EPN species, *Steinnernema carpocapsae*, and simultaneously for about another 2 decades, the same trend existed where several imported strains of EPNs were studied mainly for biocontrol point of view (Rao and Manjunath, 1966; Yadava and Rao, 1970; Mathur *et al.*, 1971; Singh and Bardhan, 1974; Sundarababu *et al.*, 1984). However, due to poor adaptability of these strains under Indian conditions, the search for indigenous strains to suit to the agro-climatic conditions prevailing in the country was felt necessary. Accordingly, local surveys for EPNs were undertaken from ecologically diverse geographical regions of India. However, in many cases either the isolated EPN could either be identified only up to genus level or their biocontrol potentials were mainly tested under laboratory conditions. Sivakumar *et al.* (1989) conducted survey and reported the presence of *H. bacteriophora* in Burliar, the Nilgiris. Poinar *et al.* (1992) described one new species, *H. indica* from Tamil Nadu. Singh *et al.* (1992) surveyed the soils in the centre of International Crops Research Institute for the Semi-Arid

Tropics, Patancheru (Andhra Pradesh) and isolated a *Steinernema* sp. Ambika (1995) reported *H. indica* in Coimbatore and Kanyakumari districts. Josephrajkumar and Sivakumar (1997) conducted a survey in Kanyakumari district and reported the frequency of *Steinernema* spp. and *H. indica* occurrence to be 10.43 and 0.61%, respectively. Kaushal *et al.* (2000) examined 207 soil samples collected from various regions of India out of which 17 samples contained EPNs, comprising 10 isolates of *Steinernema* and seven isolates of heterorhabditis. In central northern Gujarat, 16 different EPN isolates were encountered during surveys; which comprised four species of *Heterorhabditis*, 11 of steinernematids and one unidentified species (Vyas *et al.*, 1998). In further surveys around Anand using *Galleria mellonella* as bait, 4 unidentified isolates of *Steinernema* were encountered (Ganguly *et al.*, 2002). Prasad *et al.* (2001a, b) undertook random surveys of EPNS and showed the presence of heterorhabditis in soil samples collected from various localities representing various agro eco-systems in South Andamans. Gokte-Narkhedkar *et al.* (2001) collected sixteen isolates of EPNs belonging to *Steinernema* and *Heterorhabditis* spp. from cotton growing areas of north, south and central India using *Corcya cephalonica* and *Galleria mellonella* as baits. In western ghat region of Tamil Nadu, EPNs were recorded in 7.0% samples and comprised nine isolates of steinermatids (Sivakumar *et al.*, 2002). In an extensive survey from several districts of Rajasthan, comprising 477 soil samples, *Steinernema* sp. was recorded only in three samples in Udaipur, While *Heterorhabditis* was prevalent in four samples in Udaipur and one

samples from Jaipur (Parihar *et al.*, 2002). Ganguly and Singh (2000) isolated one new species from Indian Agricultural Research Institute, New Delhi fields and described it as *S. thermophilum.* The natural occurrence of *H. indica* was also reported from Kerala by Banu *et al.* (1998) and it was also found associated to the extent of 28.0% with rhinoceros beetle, a pest of coconut (Sosama *et al.*, 2002). Banu *et al.* (2005) also reported the presence of *H. indica, S. glaseri* and one *Steinernema* sp. from Kerala.

Habitat and occurrence of EPNs:

Akhurst and Bedding (1986) reported that no significant difference occurs in the prevalence of heterorhabditids and steinernematids between forests and grasslands, but Akhurst and Brooks (1984) recorded a greater proportion (39.0%) of EPNs in fruit orchard, where high density of insect pest damage was observed. Similar observations were also made by Mracek and Webster (1993), who reported that most of the steinernematids and heterorhabditids are found in places where insect infestation is noticeable and no human influence on the habitat. Mracek *et al.* (1999) found that sampling sites with moderate to severe insect abundance were positive for EPNs to the extent of 66.5% while with slight or no visible insect abundance it was only 16.0%. Mracek and Jenser (1988) reported that the number of *N. carpocapsae* recovered from samples of experimental orchard correlated closely with those of suitable host of this nematode. Nematodes were present in soil samples of all orchard type with exception of walnut tree

stand. *H. heliothidis* was found in orchard where *Polyphylla fullo,* the host for this nematode was abundant. Bednarek and Mracek (1986) reported that EPNs were abundant in habitat where nematodes have been introduced for biological control (23 – 24 million IJs/0.5 ha) and least abundant in one of the control habitat, a mountain grassland (1.3 million IJs/0.5 ha). *H. megidis* was reported to be present in grassland (golf course) (Poinar *et al.*, 1987).

Kleespies *et al.* (1989) reported the presence of higher population of steinernematids and heterorhabditids in organic fields and orchards than from conventional ones. Roadside verges harbour steinernematids most often while healthy land sites yielded them often less (Hominick and Briscoe, 1990). Hara *et al.* (1991) observed higher populations of heterorhabditids in ocean beaches within 100 m of seashore (0 m elevation). Griffin *et al.* (1991) found *S. feltiae* in woodland, tilled field, pasture and roadside verges while *S. affine* was absent in woodland. *S. feltiae* was found mostly in pasture and not from healthy land. Their prevalence of less than 5% in different habitats made it difficult to draw any conclusion (Boag *et al.*, 1992). Steiner (1996) reported that *S. feltiae* was confined to grassland.

Zhang *et al.* (1992) and Steiner (1994) also observed that the nematode incidence was not affected by habitat. Amarasinghe et al. (1994) noted that the presence of heterorhabditids and steinernematids were restricted to sandy coastal soils. Similarly, Griffin *et al.* (1991) reported the presence of *Heterorhabditis* sp. in 14.5% of coastal sites in Ireland and Britain and not from 40 inland sampling sites.

Heterorhabditids sp. was detected within a few hundred meters from sea. Shamseldean and Abd-Elgawad (1994) reported that the heterorhabditids are recovered more commonly from soils planted with mango trees and vegetation free sites. Josephrajkumar and Sivakumar (1997) reported that *Steinernema* sp. was found higher in numbers in hilly region receiving high rainfall than along the coastal belt and inland regions. Glazer *et al.* (1996) reported high density of heterorhabditids in plots with 50 – 75% shade, which were also characterized by high weed density. Mracek *et al.* (1999) also reported higher number of EPNs positive samples from trees than open habitat. Sturhan (1996) reported that prevalence of EPNs were highest in woodland (50.5%) and lowest in other habitat ranging from 25.6% to 33.8%. Pino *et al.* (1996) recovered EPNs from cropland soils in large number than from woodland and pasture soils. However, the difference between habitat type and EPN population was not significant. Miduturi *et al.* (1996a) reported that *S. feltiae* and *S. affine* were detected in woodland (3.3%), roadside verges (2.2%) and in grassland (0.5%) but not in cultivated fields.

Miduturi *et al.* (1997) recovered EPNs in 38.1, 28.5, 23.8 and 9.6% of samples drawn from woodlands, roadside verges, cultivated land and grassland, respectively. All types of *S. feltiae* were isolated in all the habitats except in the roadside verges whereas *S. affine* was not found in cultivated land. *Steinernema* sp. was isolated from cultivated fields and roadside verges while *H. megidis* from grassland only. *S. feltiae* was found to occur both in forest pasture and crop land habitats

(Barker and Barker, 1998). Yoshida *et al.* (1998) reported the presence of *H. indica* in coastal region from the subtropical to the warm temperate zones widely and *H. megidis* mainly in the warm temperate coastal region. Constant *et al.* (1998) observed the prevalence of EPNs in coastal (91.4%), tropical lowland (5.7%) and tropical middle (2.9%) altitude areas. No EPN was recorded in mountain regions. Sturhan and Liskova (1999) reported the presence of *S. affine* in arable soil, *S. intermedium* in woodland and riverbank having trees vegetation. In Indonesia, *Heterorhabditis* sp. and *Steinernema* sp. were recovered only from coastal sites (Griffin *et al.*, 2000).

Soil Type and occurrence of EPNs:

Burman *et al.* (1986) reported that steinernematids are found in all types of soils but are predominantly associated with sandy soil in Sweden. However, Blackshaw (1988) reported more occurrence of *N. bibionis* in loamy soil (85.4%) followed by clay and clayey loam (9.8%) in Northern Ireland. Nematodes were also recovered from peat soil (4.9%). Hominick and Briscoe (1990) found that *S. feltiae* was associated with calcareous soils in England. Griffin *et al.* (1991) recovered high frequency of nematodes, *Heterorhabditis* sp., *S. affine* and *S. feltiae* from sandy and peat soils than from clay and clayey loam soils. Hara *et al.* (1991) reported that the occurrence of heterorhabditids closely correlates with ocean beaches containing sand grains from coral and shell with a pH of 8.0 and low organic matter content whereas steinernematid recorded from inland areas in silty clay and silt loam

soils with high organic matter content. Hominick and Briscoe (1990) concluded the soil enriched by humus with high organic matter harbored more EPNs. Rueda *et al.* (1993) reported that the recovery of *H. bacteriophora* was more frequent from sandy loam soils (59.0%) followed by silt loam (18.0%). In contrast, *S. carpocapsae* occurred most frequently in silt loam (35.0%) followed by clay loam (29.0%) and sandy loam (29.0%). In India, Singh *et al.* (1992) reported steinernematids from soil collected from vertisols. Josephrajkumar and Sivakumar (1997) observed that probability of finding the EPN was 10 times greater in loam than in sandy soil and 5 times greater when compared to clayey and silty soils. Steiner (1994) reported *S. kraussei* as predominant EPN in soils with relatively low pH. *Heterorhabditids* were equally abundant in turf and weedy habitat, but in closed canopy forest, no occurrence of this nematode was reported (Stuart and Gaugler, 1994).

Miduturi *et al.* (1996a; 1996b) reported the largest number of *S. feltiae* in samples with sand fraction content more than 90.0% whereas *H. megidis* was detected in sand fraction more than 94.0%. *Steinernema apuliae* has been found in soil samples collected along a salt pan border habitat in southern Italy characterized by a salted silt soil (Triggiani *et al.*, 2004). Mracek *et al.* (1999) reported that EPNs were frequently found in light than heavy soils. In Korea, *Steinernema* spp. and *Heterorhabditis* spp. were mostly recorded from sandy loam soils (Lee *et al.*, 1996). In contrary to the above, Ambika (1995) reported that nematodes occurrence was not related with soil type.

Materials and Methods:

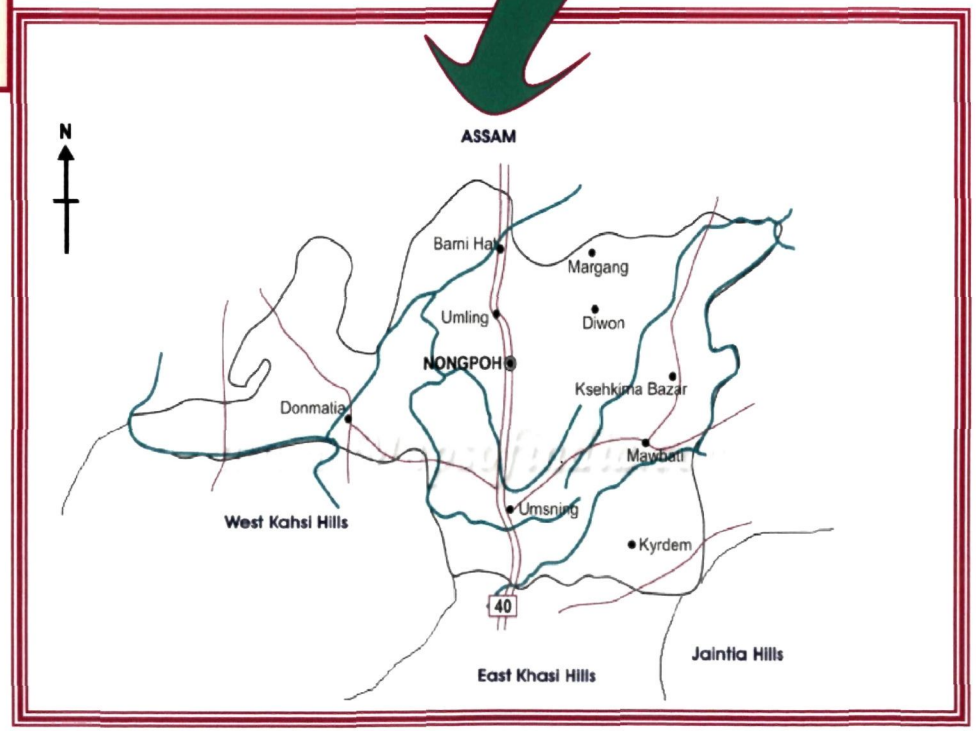
Study area

Ri-Bhoi District is one of the Seven Districts of Meghalaya, India having an area of 2448 Sq. Kms (Fig. 1.2). It lies between 91' 40' 16" Latitude and 25' 40' to 26' 20' N Longitude. This District is characterized by rugged and irregular land surface. It includes a series of hill ranges, which gradually sloped towards the North and finally joins the Brahmaputra Valley. It is bounded in the North by the Kamrup District of Assam, East by the Karbi Anglong District of Assam, South by the East Khasi Hills and West by the West Khasi Hills District.

Soil in Ri-Bhoi District may be broadly classified into hill and plain soils. Patches of red loamy soil and lime silt constitutes the major portion. This soil is much suitable for growing both local and improved varieties of crops. The soil in the adjoining Assam also consists of heavy loams while the soils in other areas are interspersed with stones and chips.

Ri-Bhoi district experiences different types of climate ranging from tropical climate in the areas bordering Assam to the temperate climate adjoining the East Khasi Hills District. The areas bordering Assam experience hot - humid weather during summer seasons with an average temperature of 30°C, especially during the month of May to July of the year. In other areas like Lum Raitong and Lum Sohpetbneng Plateaus, the climate is severely cold during the winter months and is pleasant during the summer period.

Fig.1.2: MAP OF RI-BHOI DISTRICT, MEGHALAYA.



NEMATODE SOURCE:

Surveying for nematodes

Soil samples were collected from different localities from Ri-Bhoi District Meghalaya. The samples were collected from different habitats *viz.* Dry land, Wet land, Jhum land and Forest land, at a depth of 10 – 15 cms at each site covering an area about 1 sq. m, pooled and made up to 500 gms and transported in polyethene bags to laboratory. Information on sampling months, location, soil type was noted for each sample.

CULTURE OF GREATER WAX MOTH, *Galleria mellonella*

The larvae of greater wax moth, *Galleria mellonella* (L.) were collected from Regional Resource Training Centre (RRTC), Umran. The larvae were maintained in their natural diets (honeycombs). Male and Female moths in the ratio of 1:1 were kept in the oviposition cage for egg laying. Honey-water mixture 1:1 and drinking water was provided on cotton swabs and changed daily. The cages were kept at 30°C in a dark place. The eggs were transferred to the artificial diet and reared as described by Singh (1997).

Diet ingredients for larvae of *G. mellonella*

A	Corn meal	400 gms	**B**	Honey	350 ml
	Wheat Flour	200 gms		Glycerin	350 ml
	Wheat Bran	200 gms			
	Milk Powder	200 gms			
	Yeasts Tablets	100 gms			

Preparation of the diet:

Corn meal, Wheat Flour, Wheat Bran, Milk Powder and yeasts tablets (A) were mixed thoroughly. Honey and Glycerin (B) were mixed separately. A and B were mixed slowly by thorough stirring with glass rod. The diet was prepared in a plastic container 4 or 5 days before used.

Baiting of soil samples

Nematodes were isolated by baiting techniques (Bedding and Akhurst, 1975) where the samples were baited in 500 ml container. Ten numbers of last instar larvae of wax moth *Galleria mellonella* (L.) were used as baiting agent. Three replicates were maintained for each soil samples. Larval mortality was observed daily for 10 days.

The dead larvae were washed twice or thrice in distilled water. The dead larvae were examined for the presence of entomopathogenic nematodes by colour change and smell emanating form the dead larvae. The infected larvae were transferred to white trap for extraction.

Re- infection

Re infection of extracted nematodes was done on petriplates lined with moist filter paper and re-isolated to confirm their pathogenicity through Koch's postulates.

CULTURE OF ENTOMOPATHOGENIC NEMATODES

Cleaning and Surface sterilization of instruments

All the instruments used in the study such as forceps, needles, scalpels etc., were surface sterilized with 70% ethanol (v/v) before handling any nematode isolate. Materials such as Petri dishes were surface sterilized with 70% ethanol for each use. The glass pipettes and the syringes used were washed and dried properly in the drier before they were used for the next time.

Infection of G. mellonella larvae

Laboratory cultures of all the EPNs were maintained using final instar larvae of *G. mellonella.* Nematodes were multiplied using the methods of Dutky *et al.* (1964). The nematode suspension was applied to a double layer of Whatman #1 filter paper in a petri dish. Insect larvae were introduced to the Petri dish, which was sealed and incubated at 25^{0}C.

Extraction and Storage of Nematodes

Infected larvae were transferred to modified White traps (Woodring and Kaya, 1988) and incubated at 25 ^{0}C. At the time of infective juveniles' emergence, they were extracted in a beaker and cleaned two or three times with distilled water by sedimentation, followed by decantation. The extracted nematodes were stored at 10 ^{0}C in distilled water for future use.

All the isolates were maintained separately by following the above procedures and used for laboratory experiments.

MORPHOLOGICAL CHARACTERIZATION

Light microscopy

All nematodes used in this study were produced in *G. mellonella* larvae. Ten *G. mellonella* larvae were exposed to about 2000 infective juveniles (IJ) in a Petri dish (60 x 15 mm) lined with two moistened filter papers at 25°C. After the larvae died, the insect cadavers were transferred to a modified white trap (Woodring and Kaya, 1988) and incubated at 25 °C until IJs emerged.

First and second generation adult steinernematids were obtained by dissecting infected insects 2 to 3 days and 5 to 7 days, respectively, after the insects died while hermaphrodite and male/female of heterorhabditids were obtained by dissecting 4 to 5 days and 6 to 8 days respectively, after insects died. The infective juveniles used for measurements were collected 3 days after the first emergence of IJs. All nematode samples, including IJs, the first and second generation males and females, were killed by gentle heat and then fixed in triethanolamine–formalin (TAF) fixative (Courtney *et al.*, 1955) and processed to anhydrous glycerol using the method described by Seinhorst (1959). Permanent slides were made using glass slide; cover glass supports were used in all cases to avoid flattening of specimens. At least 50 each of female, male and infective juvenile were observed and measured. Measurements and their photography were conducted

using a Leitz-Dialux 20 EB microscope with 10x, 20x or 40x differential interference contrast lens. All measurements, taken with the help of ocular and stage micrometers are in μm and based on fifty specimens of each stage unless otherwise stated.

Scanning Electron Microscopy

For scanning electron microscopic (SEM) examination, adults and IJs were washed thoroughly in distilled water to remove any debris and fixed in Karnovsky's fixative, buffered with 0.1 M sodium cacodylate. They were post-fixed with 2% osmium tetraoxide solution for 12 hours at 25°C, dehydrated in a graded acetone series and dried with Tetramethylsilane (TMS) as described by Dey *et al.* (1989). The specimens were mounted and positioned on stubs, coated with a thin layer of gold in a Fine Coat Ion Sputter and examined using JEOL (JSM – 6360) scanning electron microscope, operating at electron accelerating voltage of 10-15 KeV.

Observations and Results

Surveying for nematodes

In the present study, the entomopathogenic nematodes were recorded from 89 samples (5.37%) out of 1656 samples collected from 20 villages representing ecologically diverse types of soils (Table 1.3). Out of 89 positive samples, the frequency of occurrence of *Steinernema* spp. was found to be more (73.03%) than *Heterorhabditis* sp. (26.97%). All the EPN positive samples were from the forest soils except the one isolated from banana plantations near teak forest in Jorabat. No

Table 1.3: Prevalence and distribution of Entomopathogenic Nematodes in Ri-Bhoi District, Meghalaya.

Habitats	No. of samples collected	No. of samples positive for EPNs			
		Heterorhabditis sp.	*Steinernema* spp.	Total	% positive sample
Dryland	480	-	-	-	-
Wetland	216	-	-	-	-
Forestland	576	24 (4.17 %)	65 (11.28 %)	89	15.45
Jhumland	384		-	-	-
TOTAL	1656	24 (1.45 %)	65 (3.93 %)	89	5.37

entomopathogenic nematodes were isolated from dryland (soil moisture less than 8.0%), jhumland (burned and cultivated land) and wet land (water-saturated soil). *Steinernema* spp. were mostly isolated from sandy loam soils while *Heterorhabditis* sp., was isolated from red loamy soil. The relationship between the presence of the nematode and soil insects was not recorded in the present study.

Based on their morphometric measurements and behavior (colour of dead insect larvae killed by the nematode, i.e., brick red in Heterorhabditids and pale yellowish in *Steinernema* spp.), the isolated nematodes were grouped into three types:

- Isolate 1 : *Heterorhabditis* sp.
- Isolate 2: *Steinernema* sp. of small IJs length.
- Isolate 3: *Steinernema* sp. of long IJs length.

Nematodes identification

The species identification of three different isolates of EPNs recovered in this study was made based on light and scanning electron microscopical studies and morphometric analysis of different stages of nematodes.

The isolate 1 recorded in this study was identified to be ***Heterorhabditis indica*** based on following salient characters:

1. Occurrence of hermaphroditic stage in their life cycle and presence of bursa in males distinguished it from the genus *Steinernema.*
2. Small average length of Ijs (542.78 µm) and ratio c (Body length/Tail length) measurement (5.48 µm) differentiate it from other described species of *Heterorhabditis.*

3. Smallest average length of male (686.91 µm)
4. Adults with six distinct protruding pointed lips that surround oral aperture. Each lip bearing one labial papilla.
5. Vulva located near middle of the body, with protruding lips.
6. Anal region of amphimictic females and hermaphrodites conspicuously swollen.

Isolate 2 was identified to be ***Steinernema thermophilum***, because of the following salient features:

1. Presence of double horn-like structure at the lip region of Ijs put the isolate in the group of *S. riobrave* along with *S. abbasi* and *S. pakistanense.*
2. However, presence of mucron in the second generation females distinguishes *S. thermophilum* from *S. riobrave, S. abbasi* and *S. pakistanense* as only *S. thermophilum* has double horn-like structure at the lip region of Ijs and mucron at the tail tip of second generation female.

Isolate 3 was identified to be ***Steinernema glaseri*** based on the following characters:

1. Long IJ length put the isolate into Glaseri group.
2. Average measurement of Ijs, ratio c (body length/tail length), d (Excretory pore/Oesophagus) and ratio e (Excretory pore/Tail length) similar with measurement of *S. glaseri.*
3. Near the level of the anus in the Ijs, the four central ridges enlarged.
4. Spicule length and ratio d (EP/ES) of first generation male similar with measurement of *S. glaseri.*

5. Vulva slightly protruding from the body surface, with a thick flap.
6. Spicule blade long and narrow bearing two ridges. The distal tip of the spicules bearing a ventral aperture which make it appear "hook- or "notch-like".

Details of the morphometric measurements of various stages of three entomopathogenic nematode species recovered in the present study are provided in Tables 1.4 – 1.8. The comparisons of the morphometric data of these EPN species with other related species in the genus are shown in Tables 1.9 – 1.17.

Description of the nematodes

Heterorhabditis indica

Class	Secernentea von Linstow, 1905
Order	Rhabditida (Orley, 1880) Chitwood, 1933
Suborder	Rhabditina (Orley, 1880) Chitwood, 1933
Superfamily	Rhabditoidea (Orley, 1880) Travassos, 1920
Family	Heterorhabditidae Poinar, 1976
Genus	*Heterorhabditis* Poinar, 1976
	H. indica Poinar, Karunakar and David, 1992 (Syn. *H. hawaiiensis* Gardner, Stock and Kaya, 1994)

(Plates: 1.1 -1.8)

Hermaphrodite females:

Head truncated or slightly rounded with six protruding lips around mouth opening. Oesophagus cylindrical, lacks a pronounced

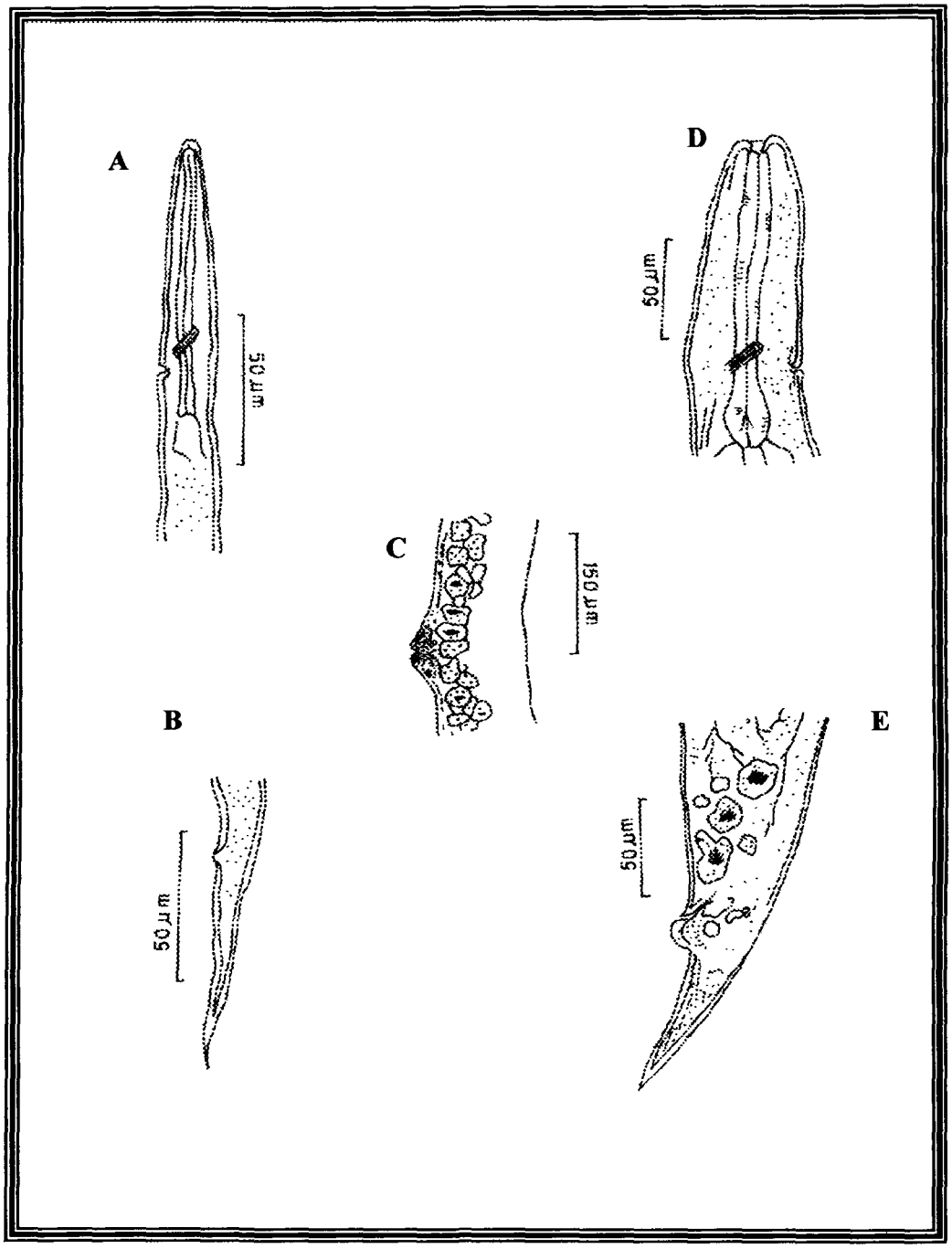


PLATE 1.1 *Heterorhabditis indica*

Figs. A-B. Infective juvenile; **A.** Anterior end showing excretory pore, oesophagous and nerve ring **B.** Posterior end showing pointed tail tip. **Figs. C-E. Hermaphrodite**; **C.** Vulvar opening **D.** Anterior end showing excretory pore, oesophagous and nerve ring **E.** Posterior end showing anal swelling.

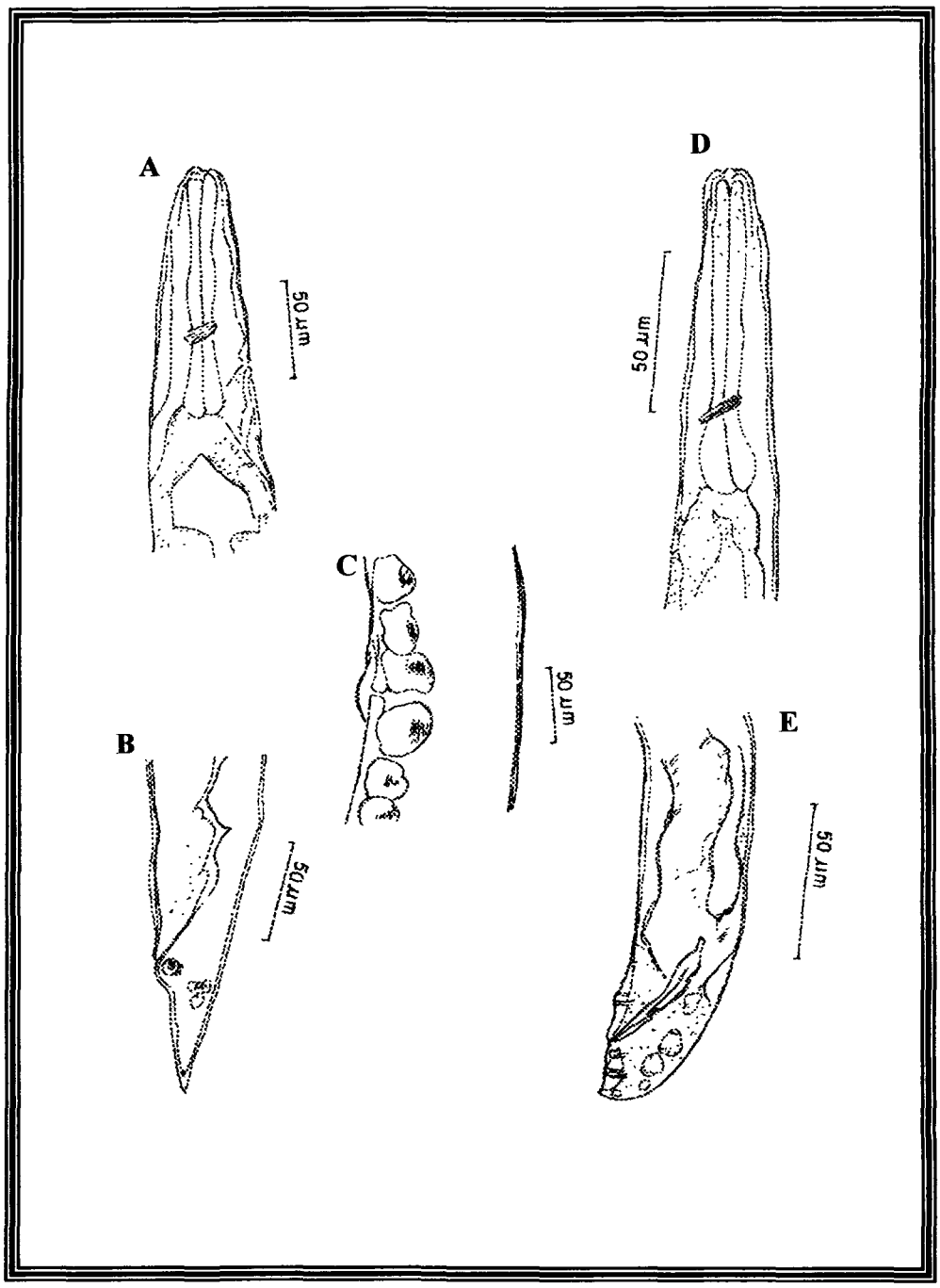


PLATE 1.2 *Heterorhabditis indica*

Figs. A-C. Amphimictic female; **A.** Anterior end showing excretory pore, oesophagous and nerve ring **B.** Posterior end showing anal swelling **C.** Vulvar opening **Figs. D-E. Male**; **D.** Anterior end showing oesophagous and nerve ring **E.** Posterior end showing spicules with bursa.

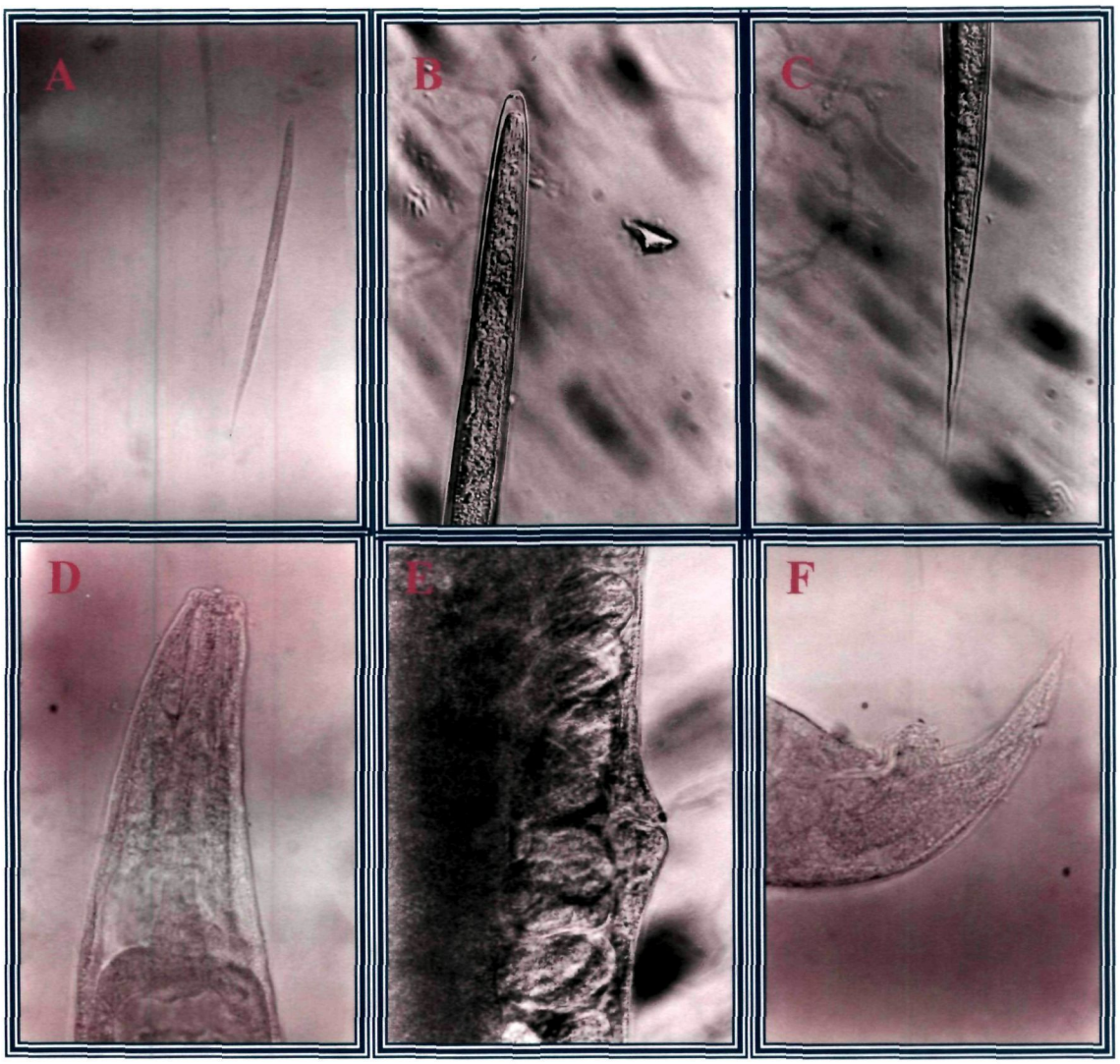


PLATE 1.3 *Heterorhabditis indica* (Photomicrographs)

Figs. A-C. Infective juvenile; **A**. Whole body* **B**. Anterior end **C.** Posterior end showing pointed tail tip **Figs. D-F. Hermophrodite**; **D**. Anterior end showing oesophagous **E.** Vulvar opening **F**. Posterior end showing anal swelling and pointed tail tip.

*(*10X, all other photographs are taken at 40X.)*

PLATE 1.3 *Heterorhabditis indica*
[Photomicrographs]

Figs. A-C. Infective juvenile A [illegible] **B** [illegible] **C.** [illegible] tip **Figs. D-F. Hermaphrodite D** [illegible] **E** [illegible] **F** Post[illegible] [illegible] tip

[illegible] photographs are taken at 40X [illegible]

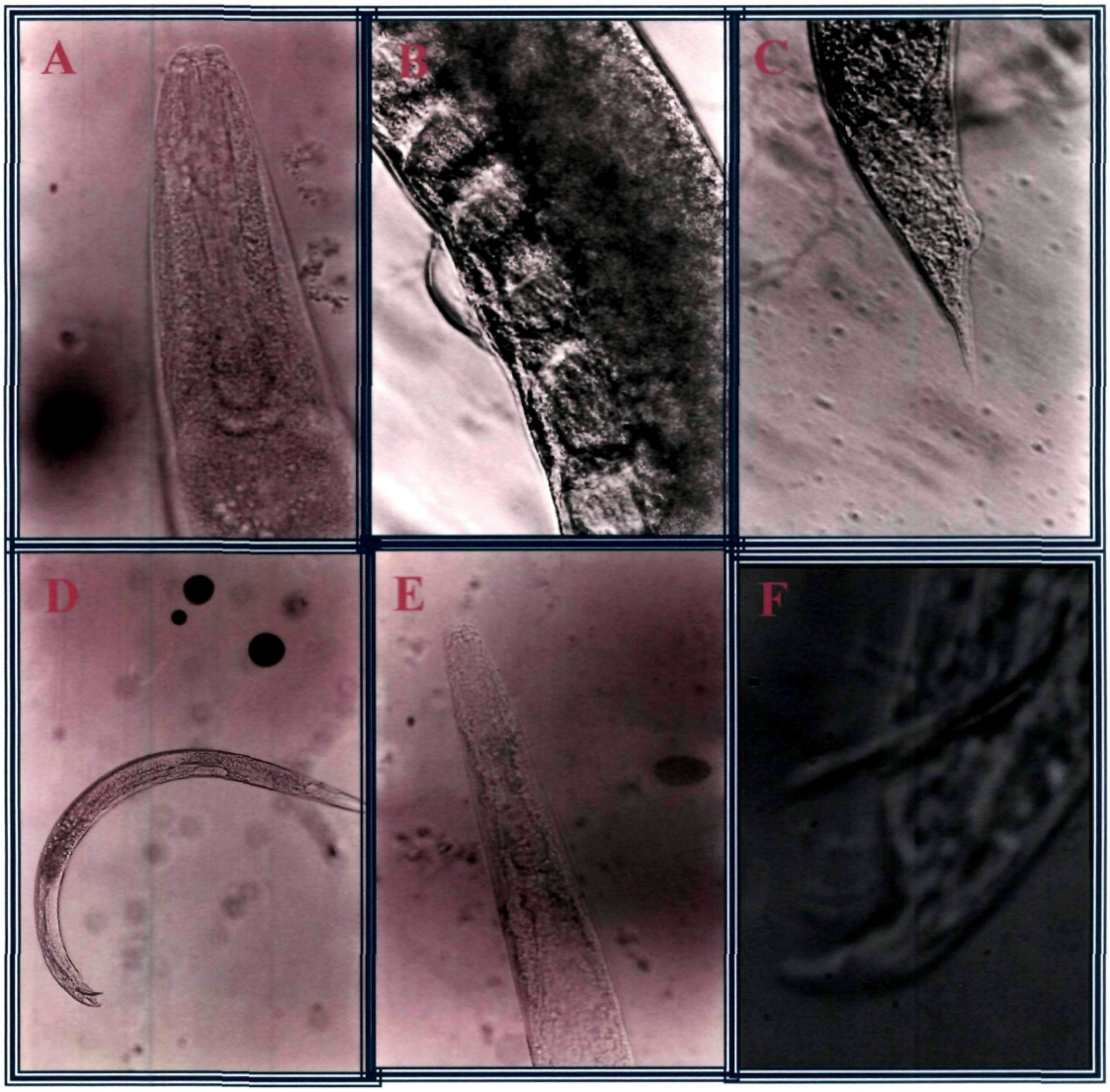


PLATE 1.4 *Heterorhabditis indica* (Photomicrographs)

Figs. A-C. Amphimictic female; **A.** Anterior end showing Oesophagus **B.** Vulvar opening **C.** Posterior end showing anal swelling **Figs. D-F. Male**; **D.** Whole body* **E.** Vulva **F**. Posterior end showing spicules with bursa**.

*(*10X, **100X, all other photographs are taken at 40X)*

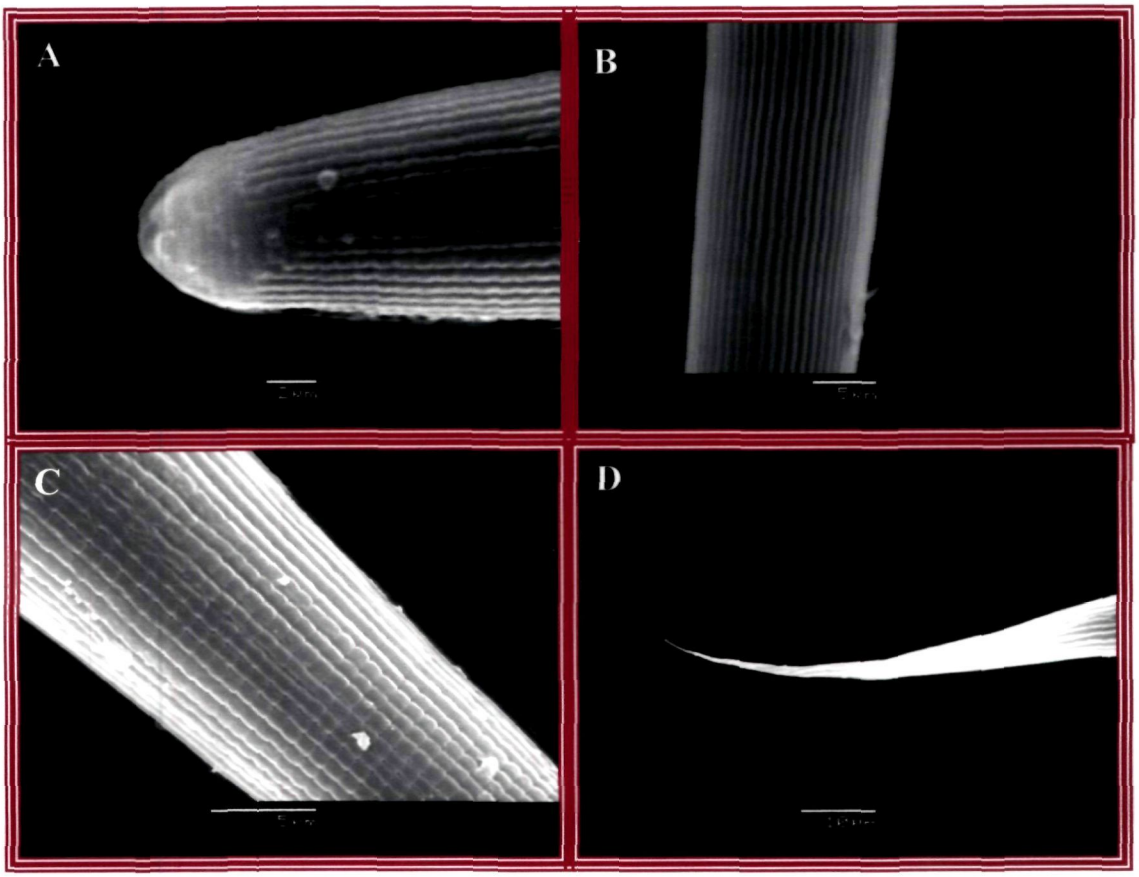


PLATE 1.5 *Heterorhabditis indica*
(Scanning electron micrographs)

Figs. A-D. Infective juvenile; **A.** Anterior end **B.** Longitudinal ridges **C.** Ventral and Longitudinal ridges **D.** Posterior end showing pointed tail tip.

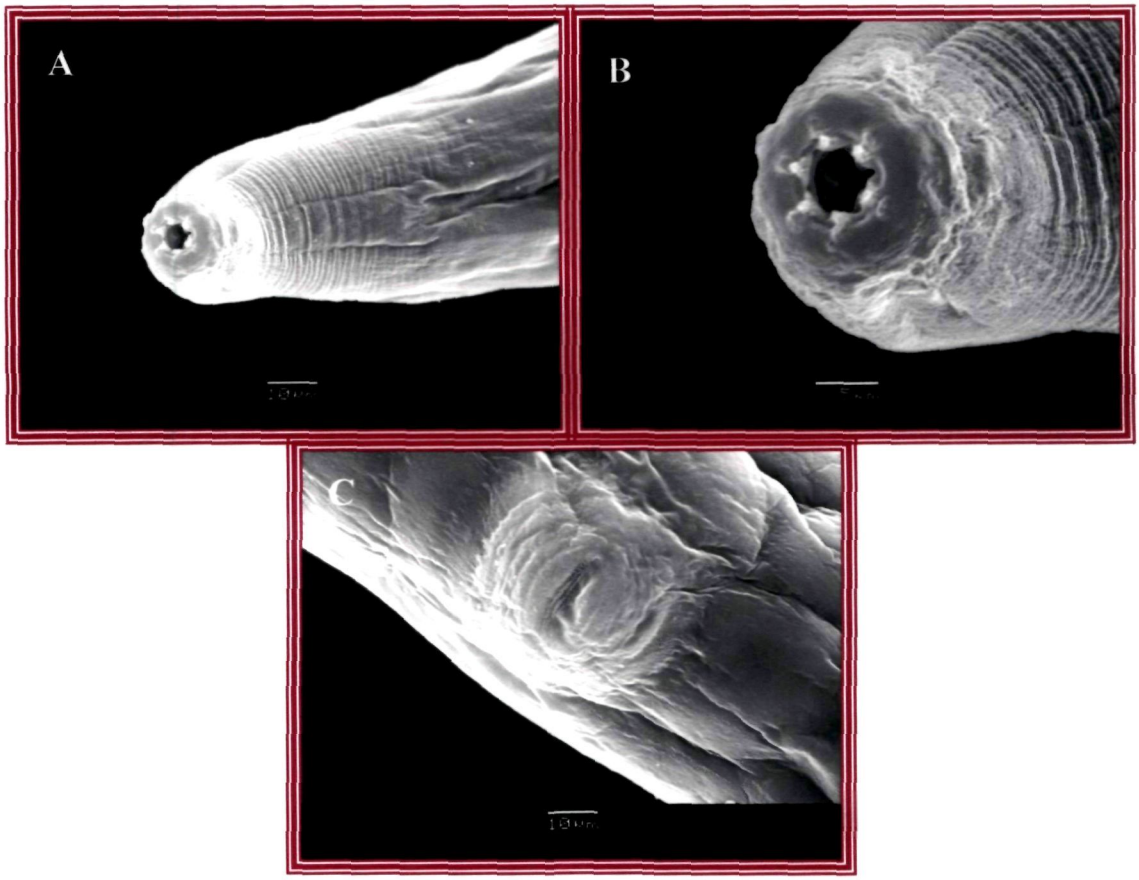


PLATE 1.6 *Heterorhabditis indica*
(Scanning electron micrographs)

Figs. A-C. Hermaphrodite; **A**. Anterior end showing mouth region **B.** Lip region showing leaf like papillae **C**. Vulvar opening.

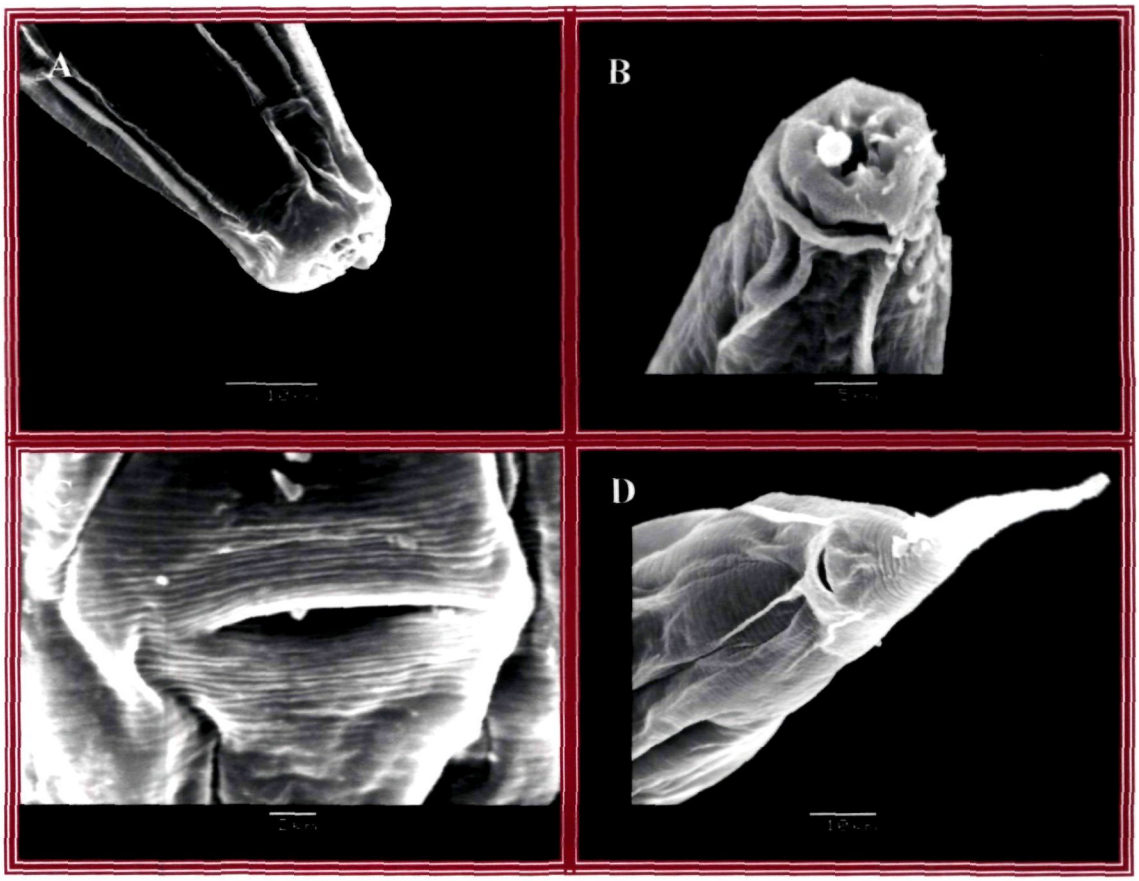


PLATE 1.7 *Heterorhabditis indica*
(Scanning electron micrographs)

Figs. A-D. Amphimictic female; **A.** Anterior end **B.** Anterior end (en face view) **C.** Vulvar opening **D.** Posterior region showing anal opening and tail.

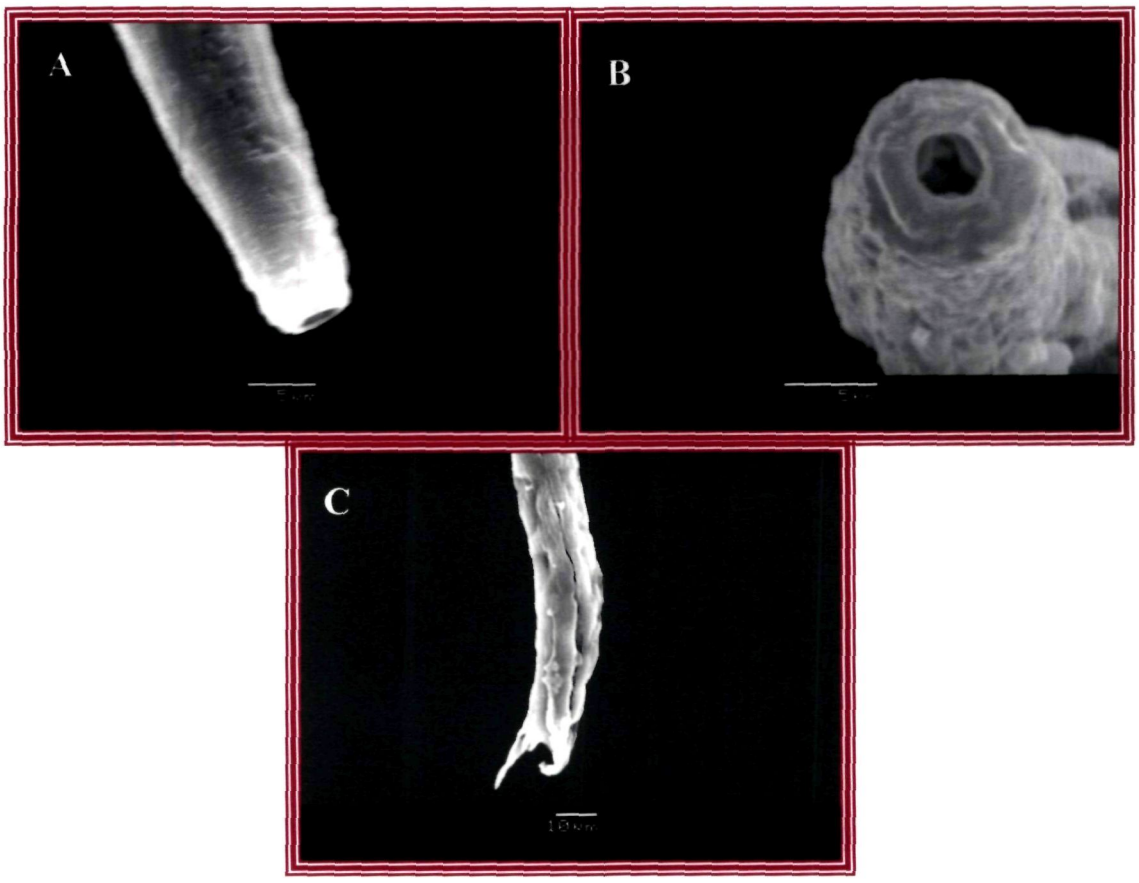


PLATE 1.8 *Heterorhabditis indica*
(Scanning electron micrographs)

Figs. A-C. Male; **A**. Anterior end **B.** Anterior end showing mouth opening **C.** Posterior region showing spicule.

metacarpus, containing an isthmus and distinct basal bulb. Nerve ring prominent and located in the middle of the isthmus and anterior to excretory pore. Vulval opening median in position of body length. Anal region conspicuously swollen and the anal opening located at the anterior one third of anal swelling. Rectum heavily sclerotized and a conspicuous valve separates it from intestine.

Amphimictic Females:

Head region with six distinct forward-directed, pointed lips that surround oral aperture. Each lip bearing one outwardly curved labial papilla. Gonads amphidelphic. Vulva narrow, located near middle of the body, with protruding lips; the same observed to be surrounded with copulation plug after mating. Anal region of amphimictic females conspicuously swollen. The anus approximately at the anterior third of anal swelling. Rectum heavily cuticularized with a conspicuous valve separating it from the intestine.

Males:

Anterior region similar to that of females, but smaller. Nerve ring near the basal bulb. Testis single, reflexed. Spicules paired, separate, and slightly curved ventrally with pointed tips; capitulum set off from shaft; blade with single medial rib. Gubernaculum flat, narrow, approximately half the spicule length, not reflexed at tip. Bursa open, peloderan, with a double membrane, one running external and the other internal to the bursal papillae; bursa with only seven normal

papillae, the last two (8 and 9) atrophied, highly modified (round or very slender). From anterior to posterior, bursal pair no. 1 normally located anterior to the spicule heads; pairs no. 2 and 3 forms a group located near the middle of the spicules. The fourth, fifth and sixth pairs form another group located near the cloacal opening. The fourth pair turn outward (laterally) and do not reach the bursal rim, the fifth and sixth pair turn ventrally (inward) and usually reach the inner bursal rim. The seventh pair branched or swollen at their base and variable in form (straight, bent outward or inward). The eighth and ninth pairs short, narrow and swollen.

Infective juveniles:

Infective juvenile enclosed within a sheath (cuticle of second-stage juvenile). Prominent longitudinal ridges present throughout the body. Head with a prominent dorsal tooth anteriorly. Mouth slightly open and cloaca closed. Oesophagus and intestine reduced. Stoma walls opened and not collapsed at base. Nerve ring prominent and excretory pore located beneath nerve ring. Tail pointed. Hemizonid quite distinct and located just anterior to excretory pore. The smaller less distinct hemizonion located in the anterior intestinal region.

The morphometric measurements of *H. indica* are given in Tables 1.4 – 1.8. The comparisons of the morphometric data of *H. indica* with other related species in the genus are provided in Tables 1.9 – 1.12.

Table 1.4: Morphometric measurements (MEAN ± SEM) of infective juveniles of EPNs (in μm).

	Heterorhabditis indica	***Steinernema thermophilum***	***Steinernema glaseri***
BL	**542.78 ± 3.13** (478.80 - 587.10)	**542.75 ± 6.64** (513.0 - 658.30)	**1386.09 ± 19.95** (1074.4 - 1556.1)
BW	**20.32 ± 0.15** (18.52 - 22.80)	**23.77 ± 0.31** (22.80 - 28.50)	**45.37 ± 0.79** (39.9 - 54.15)
ES	**117.25 ± 0.53** (108.30 - 125.40)	**98.04 ± 0.74** (91.20 - 102.60)	**157.21 ± 0.88** (148.20 - 165.30)
EP	**91.96 ± 0.62** (85.50 - 102.60)	**44.12 ± 0.37** (39.90 - 45.60)	**104.88 ± 1.14** (85.50 - 116.85)
NR	**79.23 ± 1.07** (76.95 - 82.65)		
ABW	**13.28 ± 0.20** (11.40 - 17.10)	**14.36 ± 0.20** (11.40 - 17.10)	**29.98 ± 0.29** (28.50 - 31.35)
TL	**97.75 ± 1.84** (94.05 - 108.30)	**52.48 ± 0.95** (39.90 – 57.00)	**89.38 ± 1.65** (68.40 - 102.60)
A	**26.75 ± 0.17** (23.88 - 28.75)	**22.86 ± 0.21** (20.00 - 25.25)	**30.68 ± 0.49** (23.56 to 34.87)
B	**4.63 ± 0.03** (4.24 - 5.10)	**5.54 ± 0.07** (5.11 - 6.42)	**8.82 ± 0.12** (6.98 - 9.80)
C	**5.48 ± 0.03** (5.06 - 6.10)	**10.42 ± 0.19** (9.25 - 12.9)	**15.62 ± 0.34** (12.86 – 20.00)
D	**0.79 ± 0.01** (0.72 - 0.87)	**0.45 ± 0.01** (0.42 - 0.5)	**0.67 ± 0.01** (0.56 - 0.71)
E	**0.93 ± 0.01** (0.84 - 1.06)	**0.85 ± 0.02** (0.70 - 1.07)	**1.19 ± 0.02** (1.03 - 1.54)
F	**0.20 ± 0.001** (0.18 - 0.23)	**0.46 ± 0.01** (0.40 - 0.57)	**0.51 ± 0.01** (0.40 - 0.66)

BL= Body length; **BW** = Body width; **ES** = Oesophagous length; **EP** = Excretory pore position from anterior end; **NR** = nerve ring from anterior end; **ABW** = Anal body width; **TL** = Tail length; **A** = BL/BW; **B** = BL/ES; **C** = BL/TL; **D** = EP/ES; **E** = EP/TL; **F** = BW/TL

Table 1.5: Morphometric measurements (MEAN ± SEM) of first generation female of EPNs.

	Heterorhabditis Indica*	***Steinernema thermophilum***	***Steinernema glaseri***
BL	**3075.09 ± 51.94** (2280 - 3933)	**4190.87 ± 185.11** (3063.75 - 6099)	**5424.39 ± 251.39** (4212.3 - 7182)
BW	**160.46 ± 2.40** (119.70 - 190.95)	**170.86 ± 2.96** (148.20 - 210.90)	**229.90 ± 6.85** (202.5 - 276.45)
ES	**175.9 ± 1.13** (159.60 - 190.95)	**168.15 ± 2.90** (151.05 - 216.60)	**275.03 ± 4.08** (259.35 - 302.10)
EP	**163.78 ± 1.38** (142.50 - 182.40)	**83.46 ± 2.34** (62.70 - 99.75)	**177.41 ± 5.67** (139.65 - 208.05)
NR	**118.75 ± 1.20** (114.00 - 122.50)		**182.4 ± 11.52** (159.6 - 196.65)
ABW	**54.67 ± 0.78** (37.05 - 65.55)	**62.29 ± 0.99** (51.30 - 71.25)	**73.15 ± 2.63** (59.85 - 85.50)
TL	**75.92 ± 1.66** (51.30 - 105.45)	**30.81 ± 0.51** (25.65 - 34.20)	**51.06 ± 1.51** (42.75 – 57.00)
D		**0.51 ± 0.01** (0.39 - 0.61)	**0.64 ± 0.54** (0.52 - 0.73)
E		**2.74 ± 0.09** (1.83 - 3.50)	**3.48 ± 0.02** (2.88 - 3.94)
V%		**52.43 ± 0.63** (47.79 - 57.64)	**55.39 ± 0.09** (52.57 - 58.32)

*Hermaphrodite female
V% = Percentage of vulval position to body length

Table 1.6: Morphometric measurements (MEAN ± SEM) of second generation female of EPNs.

	Heterorhabditis Indica*	***Steinernema thermophilum***	***Steinernema glaseri***
BL	**1488.17 ± 27.18** (1199.85 – 2109.00)	**2567.82 ± 187.45** (2137.50 - 4349.10)	**2815.6 ± 105.04** (2399.7 – 4959.00)
BW	**81.23 ± 1.91** (59.85 - 116.85)	**161.03 ± 7.41** (122.55 - 199.50)	**151.27 ± 4.29** (139.65 - 182.40)
ES	**137.99 ± 0.95** (128.25 - 159.60)	**148.20 ± 4.67** (136.80 - 159.60)	**154.15 ± 2.9** (139.65 - 185.25)
EP	**123.04 ± 0.76** (111.15 - 136.80)	**68.40 ± 0.57** (59.85 - 76.95)	**74.91 ± 3.14** (59.85 - 76.95)
NR	**87.59 ± 1.05** (85.50 - 91.50)		
ABW	**23.94 ± 0.43** (19.95 – 31.35)	**51.30 ± 1.43** (31.35 - 71.25)	**58.67 ± 1.63** (51.30 - 71.25)
TL	**62.53 ± 0.90** (48.45 - 71.25)	**28.67 ± 0.33** (25.65 - 39.90)	**27.19 ± 0.26** (25.65 - 37.05)
D	**0.89 ± 0.01** (0.73 – 1.00)	**0.46 ± 0.04** (0.35 - 0.61)	**0.51 ± 0.01** (0.39 - 0.61)
E	**1.99 ± 0.03** (1.64 – 2.58)	**2.39 ± 0.15** (1.46 - 3.50)	**2.74 ± 0.09** (1.83 - 3.50)
V%		**51.09 ± 2.15** (47.14 - 55.04	**57.03 ± 2.11** (51.54 - 59.44)

* Amphimictic female

Table 1.7: Morphometric measurements (MEAN ± SEM) first generation male of EPNs.

	Steinernema thermophilum	***Steinernema glaseri***
BL	**1057.92 ± 27.69** (983.25 - 1140)	**1413.32 ± 31.26** (1291.05 - 1573.20)
BW	**88.35 ± 1.81** (85.5 - 94.05)	**69.26 ± 2.95** (48.45 - 82.65)
ES	**128.25 ± 2.89** (116.85 - 133.95)	**165.3 ± 5.09** (136.8 - 190.95)
EP	**76.95 ± 0.91** (74.10 - 79.80)	**117.99 ± 5.41** (91.2 - 145.35)
NR	**88.35 ± 1.23** (85.5 - 96.90)	
ABW	**37.62 ± 0.57** (37.05 - 39.90)	**42.18 ± 1.19** (34.20 - 48.45)
TL	**28.5 ± 0.91** (25.65 - 31.35)	**37.62 ± 1.02** (34.20 - 42.75)
D	**0.61 ± 0.02** (0.57 - 0.68)	**0.71 ± 0.02** (0.60 - 0.81)
E	**2.71 ± 0.11** (2.45 to 3.00)	**3.16 ± 0.18** (2.40 - 4.25)
SPL	**62.7 ± 0.91** (59.85 - 65.55)	**73.53 ± 3.15** (59.85 - 94.05)
SW	**1.67 ± 0.03** (1.57 - 1.77)	**1.75 ± 0.07** (1.40 - 2.20)
GL	**31.35 ± 0.02** (31.35 - 34.20)	**44.18 ± 1.14** (39.9 - 48.45)

SPL = Spicule length; SW = SPL/ABW;
GL = Gubernaculum length

Table 1.8: Morphometric measurements (MEAN ± SEM) of second generation male of EPNs.

	Heterorhabditis indica	***Steinernema thermophilum***	***Steinernema glaseri***
BL	**686.91 ± 10.79** (521.55 – 798.00)	**847.34 ± 10.74** (726.75 – 969)	**1163.56 ± 19.51** (1054.50 - 1259.70)
BW	**41.54 ± 0.27** (37.05 - 45.60)	**56.76 ± 0.79** (45.60 - 65.55)	**55.84 ± 0.74** (51.30 - 59.85)
ES	**108.98 ± 0.59** (96.9 - 119.70)	**148.2 ± 4.67** (136.8 - 159.60)	**172.66± 4.44** (156.75 - 216.60)
EP	**114.15 ± 0.9** (99.7 -128.20)	**68.4 ± 0.57** (59.85 - 76.95)	**114.71 ± 3.08** (96.90 - 139.65)
NR	**23.11 ± 0.23** (19.95 - 25.65)		
ABW	**28.15 ± 0.23** (25.56 - 31.35)	**31.3 ± 1.43** (31.35 - 71.25)	**37.05 ± 0.86** (31.35 - 42.75)
TL	**28.15 ± 0.23** (25.56 - 31.35)	**28.67 ± 0.33** (25.65 - 39.90)	**33.96 ± 0.74** (31.35 - 37.05)
D	**1.05 ± 0.01** (0.95 - 1.17)	**0.54 ± 0.04** (0.5 - 0.63)	**0.67 ± 0.01** (0.58 - 0.74)
E	**4.08 ± 0.04** (3.50 – 4.67)	**2.47 ± 0.07** (2.00 - 2.80)	**3.39 ± 0.12** (2.69 - 4.08)
SPL	**42.64 ± 0.39** (37.05 – 54.15)	**53.14 ± 1.04** (51.30 - 62.70)	**67.01 ± 1.4** (54.50 - 71.25)
SW	**1.84 ± 0.02** (1.18 - 2.29)	**1.43 ± 0.15** (1.24 - 1.75)	**1.82 ± 0.05** (1.46 - 2.09)
GL	**19.91 ± 0.77** (17.10 - 22.80)	**31.13 ± 0.94** (25.65 - 34.20)	**35.86 ± 0.74** (31.35 - 39.9)

Table 1.9: Morphometric comparision of infective juveniles of *Heterorhabditis indica* with other related EPN species (All measurements are in µm).

	H. indica	*H. hawaiiensis*	*H. brevicaudis*	*H. bacteriophora*	*H. marelatus*	*H. argentinensis*	*H. zealandica*	*H. megidis*
BL	**542.78 ± 3.13** (478.8 - 587.10)	**575** (506 – 631)	**572** (528 – 622)	**588** (512 – 670)	**654** (540 – 700)	**657** (610 – 710)	**685** (570 – 740)	**768** (736 – 800)
BW	**20.32 ± 0.15** (18.52 - 22.80)	**25** (21 – 28)	**23** (21 – 28)	**23** (18 – 31)	28 (24 – 32)	31 (24 – 38)	27 (22 – 30)	**29** (27 – 32)
StL	**8.64 ± 0.05** (8.55 - 9.98)							
StW	**4.29 ± 0.45** (2.85 - 5.70)							
ES	**117.25 ± 0.53** (108.30 - 125.4)	**133** (115 – 181)	**124** (120 – 136)	**125** (100 – 139)	**133** (121 – 139)	**132** (101 – 150)	**140** (135 – 147)	**155** (147 – 160)
EP	**91.96 ± 0.62** (85.50 - 102.60)	92 (79 – 103)	**111** (104 – 116)	**103** (87 – 110)	**102** (81 – 113)	**107** (68 – 122)	**112** (94 – 123)	**131** (123 – 142)
NR	**79.23 ± 1.07** (76.95 - 82.65)	**114** (95 – 132)	**101** (96 – 104)	**85** (72 – 93)	**99** (83 – 113)	**95** (82 – 116)	**100** (90 – 107)	**109** (104 – 115)
ABW	**13.28 ± 0.20** (11.4 - 17.10)							
TL	**97.75 ± 1.84** (94.05 - 108.30)	**90** (82 – 108)	**75.5** (68 – 80)	**98** (83 – 112)	**107** (99 –117)	**84** (70 – 105)	**102** (87 – 119)	**119** (112 – 128)
A	**26.75 ± 0.17** (23.88 - 28.75)	**23** (22 – 24)		**25** (17 – 30)	**24** (21 – 29)	**21** (18 – 25)	**25** (24 – 26)	**26** (23 – 28)
B	**4.63 ± 0.03** (4.24 - 5.10)	**4.3** (3.5 – 4.4)		**4.5** (4 – 5.1)	**5** (4.7 – 5.4)	**5.8** (4.7 – 6.8)	**4.9** (4.2 – 5.0)	**5.0** (4.6 – 5.0)
C	**5.48 ± 0.03** (5.06 - 6.10)	**6.1** (5.8 – 6.4)	**7.6** (6.6 – 8.6)	**6.2** (5.5 – 7)	**6** (5.5 – 6.6)	**7.8** (6.7 – 8.7)	**6.6** (6 – 6.7)	**6.5** (6.1 – 6.9)
D	**0.79 ± 0.01** (0.72 - 0.87)	**0.60** (0.56 – 0.68)		**0.84** (0.76 – 0.92)	**0.77** (0.6 – 0.86)	**0.78** (0.67 – 0.81)	**0.80** (0.70 – 0.84)	**0.85** (0.81 – 0.91)
E	**0.93 ± 0.01** (0.84 - 1.06)	**1.2** (1.1 – 1.3)	**1.6** (1.5 – 1.8)	**1.12** (1.03 – 1.30)	**0.96** (0.89 – 1.1)	**1.3** (0.9 – 1.5)	**1.08** (1.03 – 1.09)	**1.1** (1.03 – 1.2)
F	**0.20 ± 0.001** (0.18 - 0.23)	**0.26** (0.25 – 0.27)		**0.25** (0.22 – 0.36)		**0.35** (0.34 – 0.36)	**0.25** (0.24 – 0.26)	**0.25** (0.23 – 0.28)

Table 1.10: Morphometric comparision of hermaphrodite female of *Heterorhabditis indica* with other related EPN species.

	H. indica	*H. hawaiiensis*	*H. brevicaudis*	*H. bacteriophora*	*H. marelatus*	*H. argentinensis*
BL	**3075.09 ± 51.94** (2280 – 3933	**5000** (4000 – 7000)	**4330** (3550 – 5040)	**4030** (3630 – 4390)	**4500**	**6500** (5000 – 7500)
BW	**160.46 ± 2.40** (119.70 - 190.95)	**337** (270 – 376)	**250** (200 – 312)	**165** (160 – 180)	**225.4**	**360** (250 – 575)
StL	**5.70 ± 0.00** (5.7 - 5.7)	**12** (9 – 13)	**10.4** (8.8 – 12)	**8** (6 – 9)	**15.8**	**13** (10 – 16)
StW	**9.41 ± 0.29** (8.55 - 11.40)	**9** (7 – 12)	**18.2** (16 – 20)	**8** (6 – 9)	**23.8**	**10** (6 – 12)
ES	**175.9 ± 1.13** (159.60 - 190.95)	**235** (187 – 283)	**218** (192 – 240)	**197** (189 – 205)	**243.5**	**274** (235 – 300)
EP	**163.78 ± 1.38** (142.5 - 182.4)	**267** (219 – 318)	**190** (160 – 200)	**209** (189 – 217)	**287.1**	**294** (250 – 340)
NR	**118.75 ± 1.20** (114 - 122.5)	**159** (102 – 212)	**164.4** (144 – 176)	**126** (121 – 130)	**182.2**	**160** (132 – 196)
ABW	**54.67 ± 0.78** (37.05 - 65.55)	**56** (38 – 79)	**70.4** (56 – 88)	**46** (40 – 53)	**57.4**	**86** (70 – 120)
TL	**75.92 ± 1.66** (51.3 - 105.45)	**84** (67 – 98)	**86** (72 – 128)	**90** (81 – 93)	**99**	**118** (100 – 140)

Table 1.11: Morphometric comparision of amphimictic female of *Heterorhabditis indica* with other related EPN species.

	H. indica	*H. hawaiiensis*	*H. brevicaudis*	*H. bacteriophora*	*H. argentinensis*
BL	**1488.17 ± 27.18** (1199.85 – 2109)	**1800** (1300 – 2300)	**2350** (2100 – 2500)	**3500** (3180 – 3850)	**3000** (2000 – 3500)
BW	**81.23 ± 1.91** (59.85 – 116.85)	**139** (104 – 171)	**149** (128 – 168)	**190** (160 – 220)	**130** (90 – 180)
StL	**5.77 ± 0.07** (5.7 – 8.55)	**9** (6 – 12)	**6** (6 – 6)	**7** (6 – 9)	**9.5** (7 – 12)
StW	**6.93 ± 0.17** (5.7 – 8.55)	**9** (7 – 12)	**10** (10 – 10)	**7** (6 – 9)	**8** (5 – 10)
ES	**137.99 ± 0.95** (128.25 – 159.6)	**137** (110 – 153)	**154** (144 – 160)	**168** (155 – 183)	**180** (162 – 200)
EP	**123.04 ± 0.76** (111.15 – 136.8)	**153** (116 – 175)	**135** (124 – 160)	**192** (174 – 214)	**203** (105 – 240)
NR	**87.59 ± 1.05** (85.5 – 91.5)	**99** (78 - 116)	**103** (100 – 108)	**103** (93 – 118)	**114** (88 – 140)
ABW	**23.94 ± 0.43** (19.95 – 31.35)	**29** (20 – 35)	**39** (36 – 48)	**28** (22 – 31)	**45** (33 – 55)
TL	**62.53 ± 0.90** (48.45 – 71.25)	**63** (49 – 87)	**86** (76 – 92)	**82** (71 – 93)	**93** (75 – 108)
D	**0.89 ± 0.01** (0.73 – 1.00)				
E	**1.99 ± 0.03** (1.64 – 2.58)				

Table1.12: Morphometric comparision of male of *Heterorhabditis indica* with other related EPN species.

	H. indica	*H. brevicaudis*	*H. hawaiiensis*	*H. bacteriophora*	*H. marelatus*	*H. argentinensis*	*H. zealandica*	*H. megidis*
BL	**686.91 ± 10.79** (521.55 – 798)	**900** (840 – 950)	**998** (864 – 1130)	**820** (780 – 960)	**942** (805 – 1046)	**1008** (823 – 1150)	**892** (780 – 1001)	**1000** (800 – 1100)
BW	**41.54 ± 0.27** (37.05 – 45.6)	**43** (40 – 48)	**64** (50 – 83)	**43** (38 – 46)	**51** (48 – 56)	**51** (40 – 64)	**48** (46 – 53)	**47** (44 – 50)
StL	**6.04 ± 0.37** (2.85 – 11.40)	**2** (1.2 – 2)	**4** (2 – 5)	**3** (2 – 4)	**3.8** (3 – 5)	**4** (2 – 5)	**4** (2 – 5)	**3** (2 – 4)
StW	**5.89 ± 0.07** (5.70 – 7.13)	**4** (3 – 5)	**3** (1 – 3)	**2** (1 – 3)	**5.4** (4 – 6)	**2** (1 – 3)	**2** (1 – 3)	**4.5** (3 – 6)
ES	**108.98 ± 0.59** (96.90 – 119.70)	**108** (104 – 112)	**119** (100 – 150)	**103** (99 – 105)	**115** (99 – 123)	**119** (76 – 135)	**127** (115 – 134)	**128** (122 – 134)
EP	**114.15 ± 0.90** (99.75 – 128.25)	**95** (92 – 100)	**130** (71 – 146)	**121** (114 – 130)	**130** (111 – 168)	**144** (83 – 172)	**135** (129 – 148)	**156** (139 – 176)
ABW	**23.11 ± 0.23** (19.95 – 25.65)	**20** (16 – 22)	**26** (21 – 34)	**33** (22 – 35)	**23** (20 – 28)	**27** (22 – 32)	**30** (26 – 36)	**26** (22 – 31)
TL	**28.15 ± 0.23** (25.56 – 31.35)	**32** (28 – 36)	**34** (26 – 40)	**28** (22 – 36)	**31** (24 – 38)	**40** (33 – 46)	**40** (30 – 46)	**39** (35 – 43)
SPL	**42.64 ± 0.39** (37.05 – 54.15)	**47** (44 – 48)	**45** (41 – 49)	**40** (36 – 44)	**45** (42 – 50)	**49** (46 – 52)	**42** (36 – 47)	**49** (46 – 54)
GL	**19.91 ± 0.77** (17.10 – 22.8)	**19** (18 – 22)	**21** (18 – 25)	**20** (18 – 22)	**19** (18 – 22)	**20** (18 – 23)	**19** (16 – 24)	**21** (17 – 24)
D	**1.05 ± 0.01** (0.95 – 1.17)							
E	**4.08 ± 0.04** (3.5 – 4.67)							
SW	**1.84 ± 0.02** (1.18 – 2.29)		**1.73** (1.44 – 1.95)	**1.28** (1.2 – 1.34)	**1.75** (1.73 – 1.78)	**1.81** (1.6 – 2.09)	**1.35** (1.3 – 1.4)	**1.35** (1.3 – 1.4)

Steinernema thermophilum

Class	Secernentea von Linstow, 1905
Order	Rhabditida (Orley, 1880) Chitwood, 1933
Suborder	Rhabditina (Orley, 1880) Chitwood, 1933
Superfamily	Rhabditoidea (Orley, 1880) Travassos, 1920
Family	Steinernematidae Chitwood and Chitwood, 1937
Genus	*Steinernema* Travassos, 1927 *S. thermophilum* Ganguly and Singh, 2000

(Plates: 1.9 – 1.18)

Female, first generation:

Body assumes spiral posture when relaxed by gentle heat. Cuticle thin and smooth. Head almost truncated, lips fused with six labial and four cephalic papillae. Amphids pore-like, located posterior to lateral labial papillae. Stoma short and wide. Base of the stoma V-shaped surrounded by oesophageal collar. Oesophagus short in relation to body length. Procorpus almost cylindrical; metacorpus slightly swollen followed; isthmus present, basal bulb pyriform with a reduced tri-radiate valve. Cardia well developed. Nerve ring encircling isthmus just anterior to the basal bulb. Excretory pore located at the level of metacorpus, excretory duct sclerotized. Gonad amphidelphic, reflexed dorsally. Oviduct well developed and uterus located in ventral position. Vagina short with muscular walls. Vulva median with protruding lips having double flapped epiptygma. The

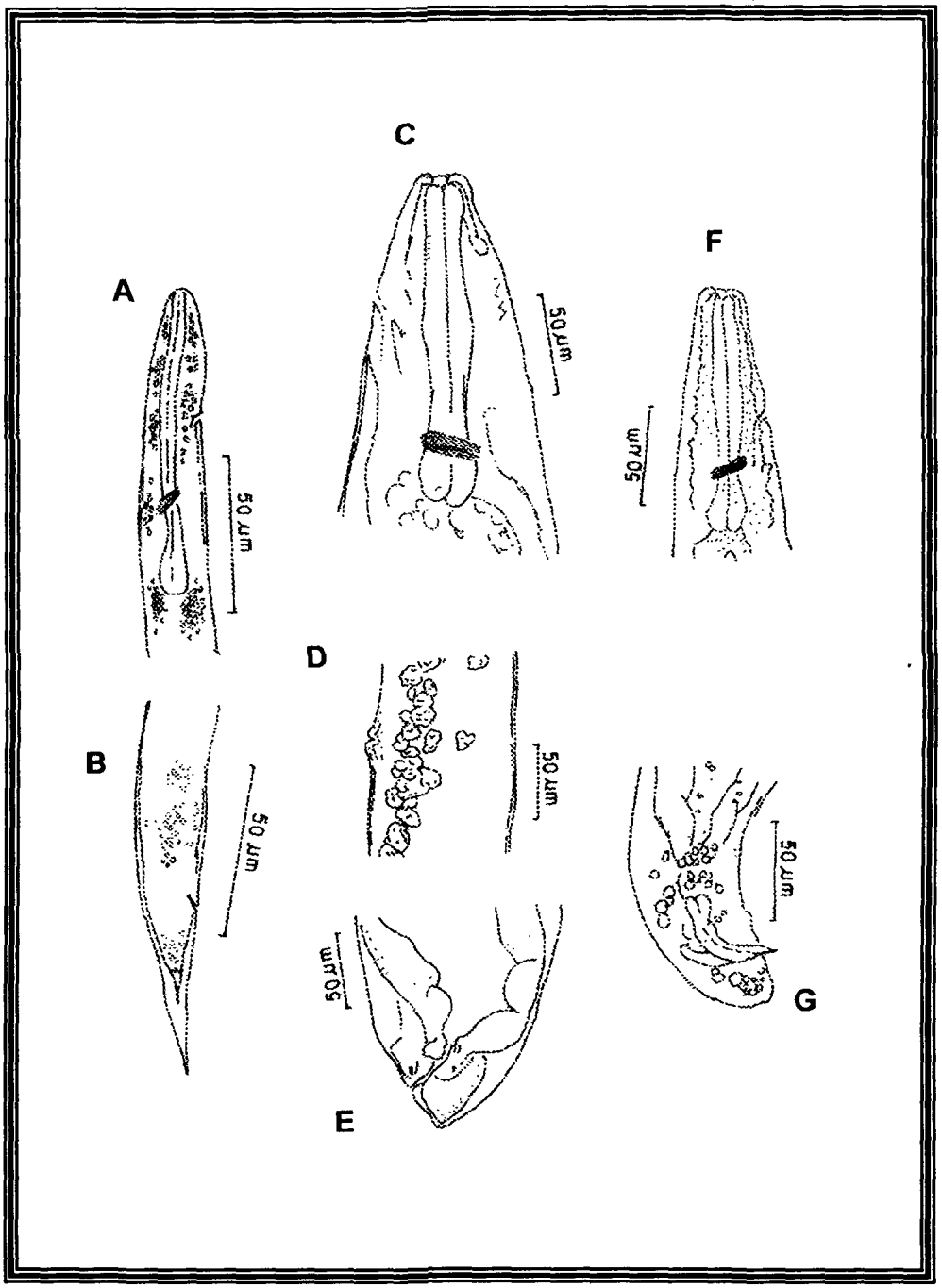


PLATE 1.9 *Steinernema thermophilum*

Figs. A-B. Infective juvenile; **A.** Anterior end showing excretory pore, oesophagous and nerve ring **B.** Posterior end showing anal pore and tail tip. **Figs. C-E First generation female**; **C.** Anterior end showing excretory pore, oesophagous and nerve ring **D.** Vulvar opening **E.** Posterior end showing anal pore **Figs.F-G. First generation male; F.** Anterior end showing excretory pore, oesophagous and nerve ring **G.** Posterior end showing spicule and gubernaculum.

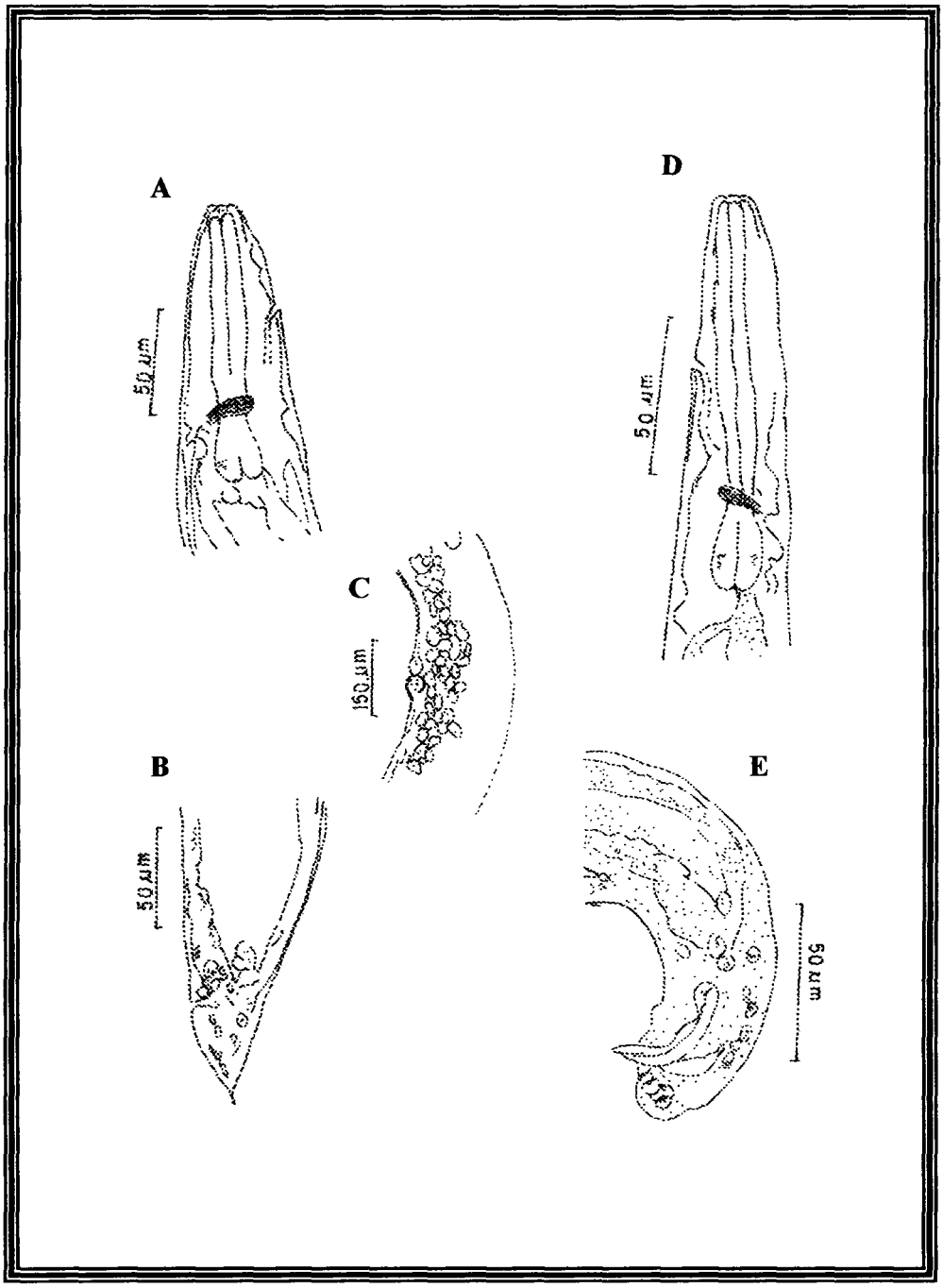


PLATE 1.10 *Steinernema thermophilum*

Figs. A-C. Second generation female; **A.** Anterior end showing excretory pore, oesophagous and nerve ring **B.** Posterior end showing mucronated tail tip **C.** Vulvar opening **Figs. D-E. Second generation male**; **D.** Anterior end showing excretory pore oesophagous and nerve ring **E.** Posterior end showing spicule and gubernaculum.

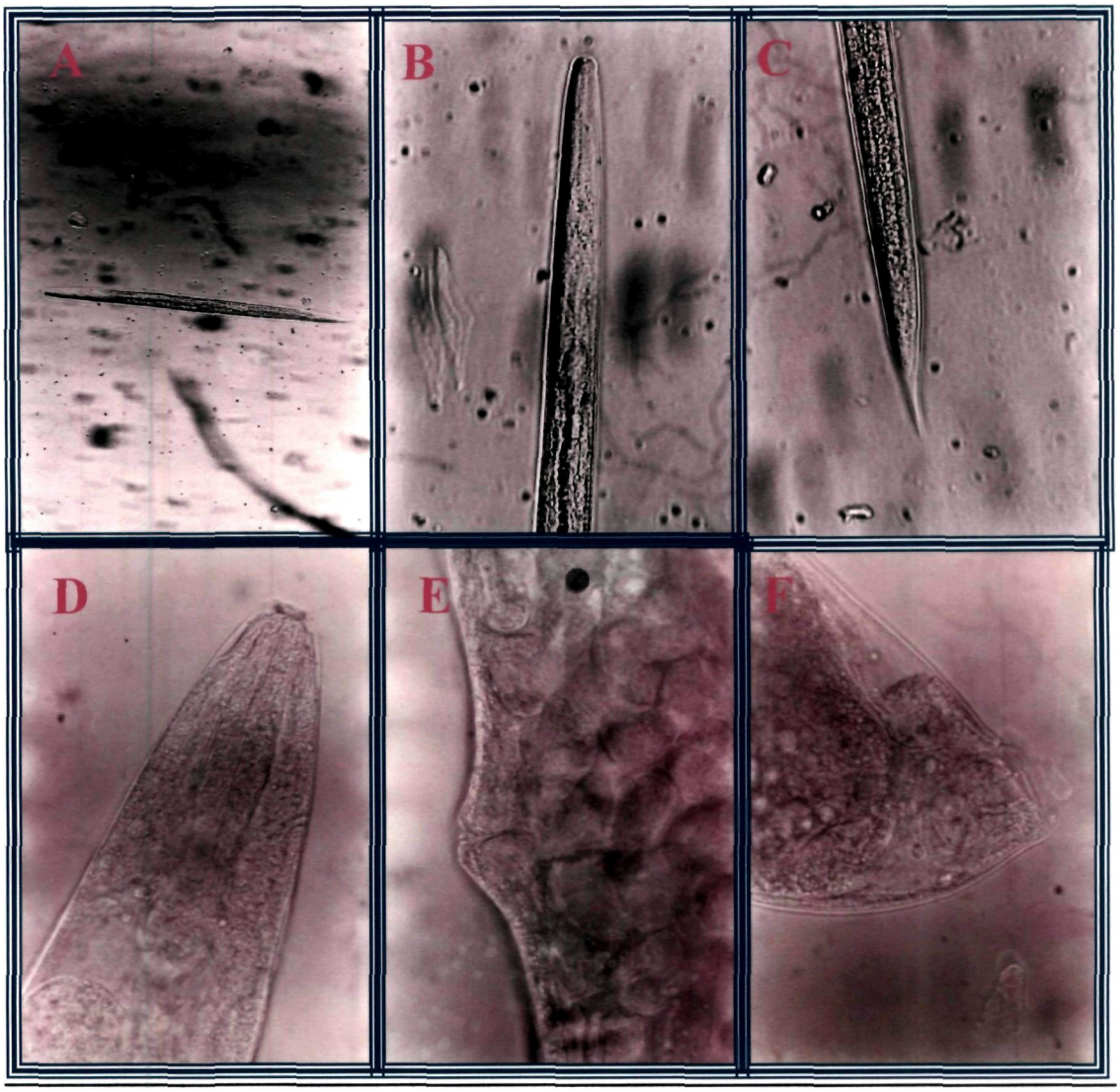


PLATE 1.11 *Steinernema thermophilum* (Photomicrographs)

Figs. A-C. Infective juvenile; **A.** Whole body* **B.** Anterior end **C.** Posterior end showing pointed tail tip **Figs. D-F. First generation female**; **D.** Anterior end showing oesophagus and excretory pore **E.** Vulvar opening **F.** Posterior end showing anal region.

*(*10X, all other photographs are taken at 40X.)*

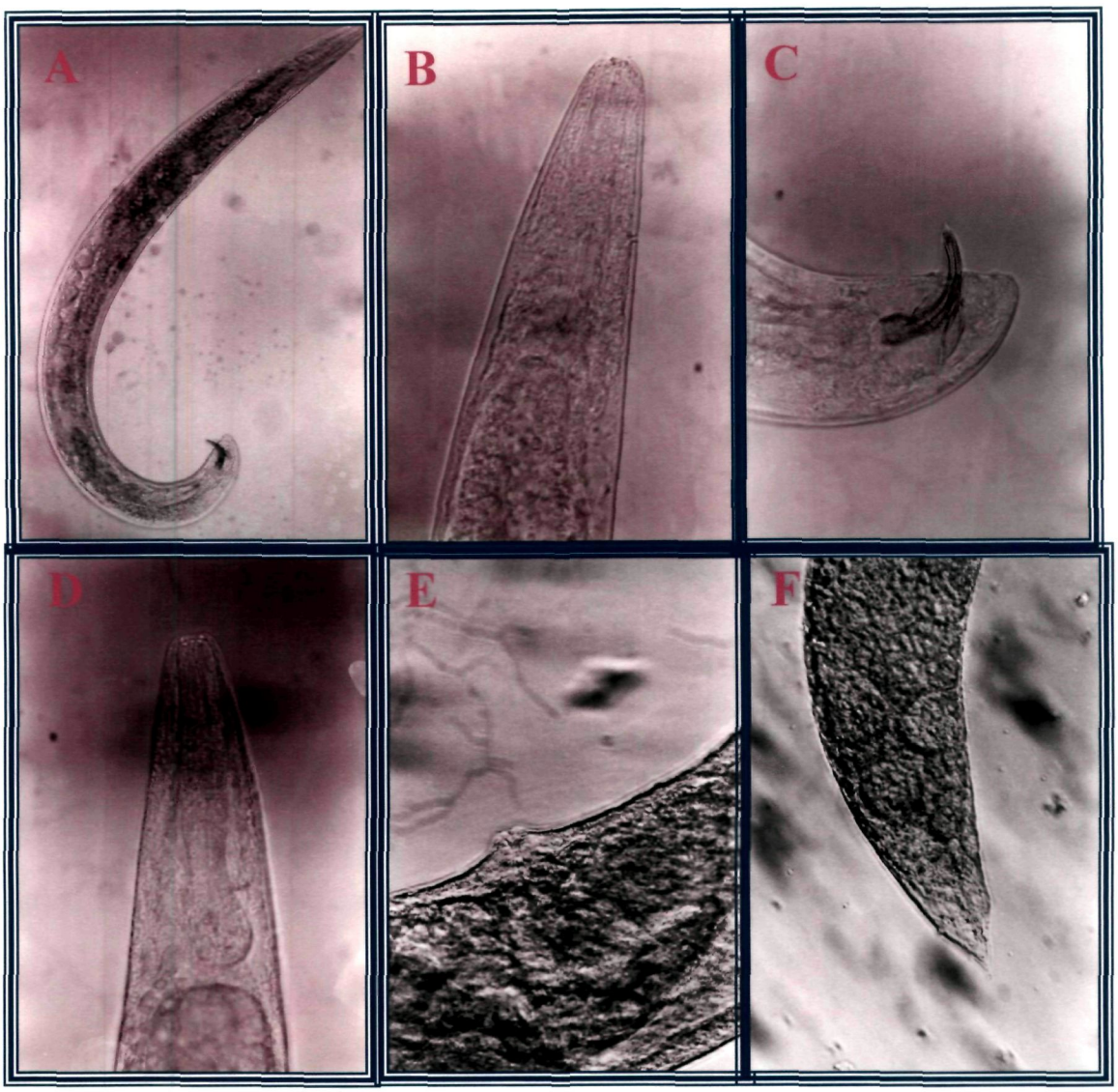


PLATE 1.12 *Steinernema thermophilum* (Photomicrographs)

Figs. A-C. First generation male; **A**. Whole body* **B.** Anterior end showing excretory pore **C.** Posterior end showing spicule and gubernaculum **Figs. D-F. Second generation female**; **D.** Anterior end showing oesophagus **E.** Vulvar opening **F.** Posterior end showing mucronated tail tip.

*(*10X, all other photographs are taken at 40X)*

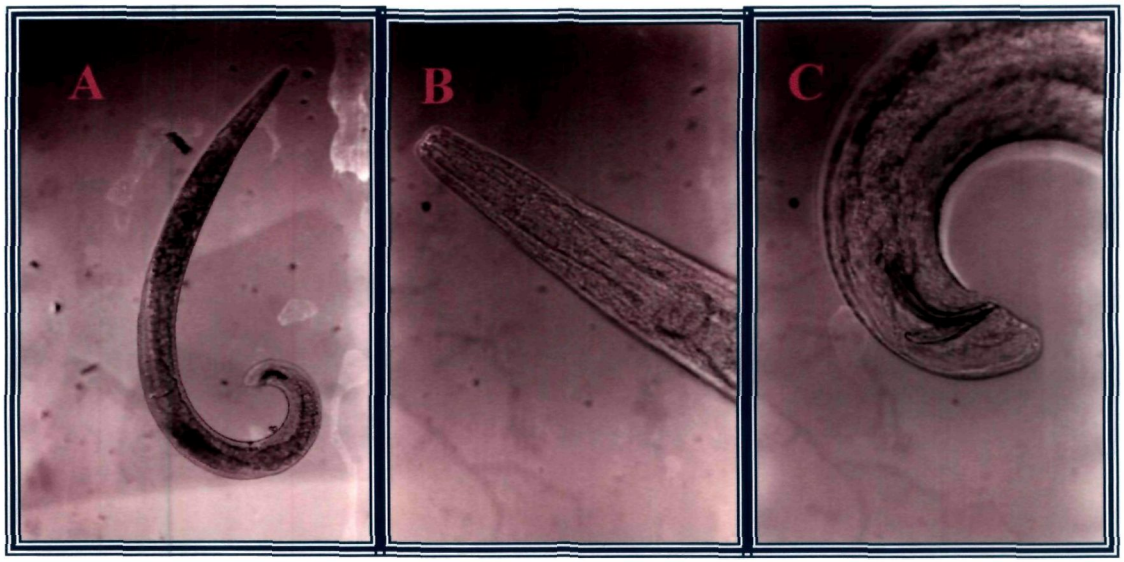


PLATE 1.13 *Steinernema thermophilum* (Photomicrographs)

Figs. A-C. Second generation male; **A.** Whole body* **B.** Anterior end showing oesophagus **C.** Posterior end showing spicule and gubernaculums.

*(*10X, all other photographs are taken at 40X)*

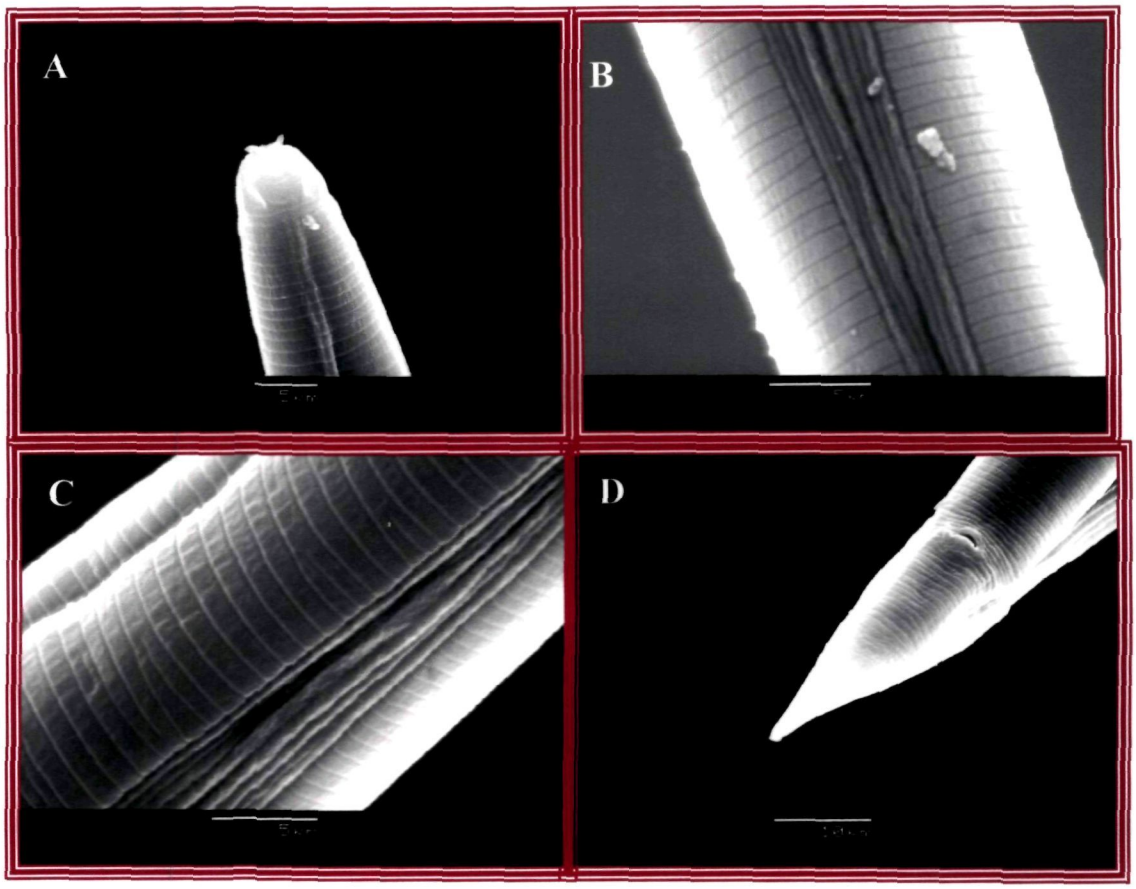


PLATE 1.14 *Steinernema thermophilum*
(Scanning electron micrographs)

Figs. A-D. Infective juveniles; **A.** Anterior end showing two horns-like structure **B.** Longitudinal ridges **C.** Ventral and longitudinal ridges **D.** Posterior end showing anal opening and tail.

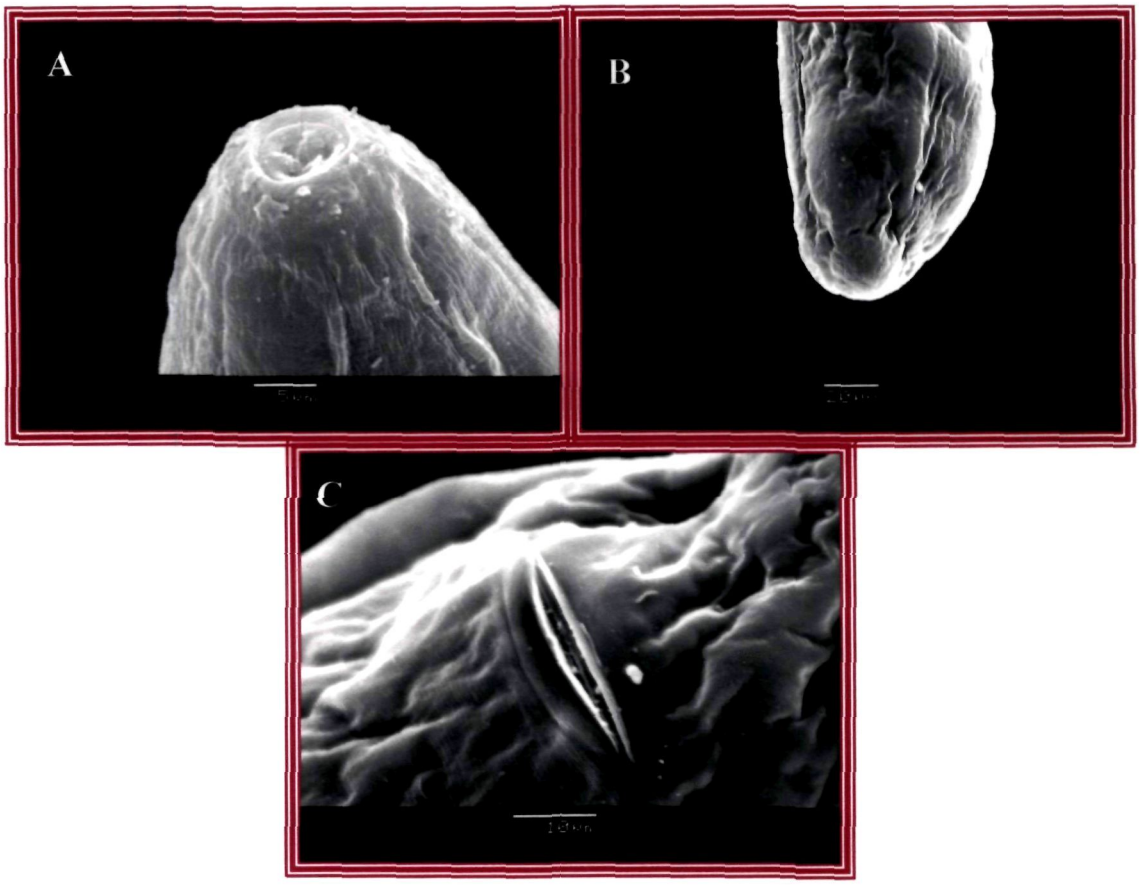


PLATE 1.15 *Steinernema thermophilum*
(Scanning electron micrographs)

Figs. A-C. First generation female; **A.** Anterior end showing mouth opening and labial papillae **B.** Posterior end **C.** Vulvar opening.

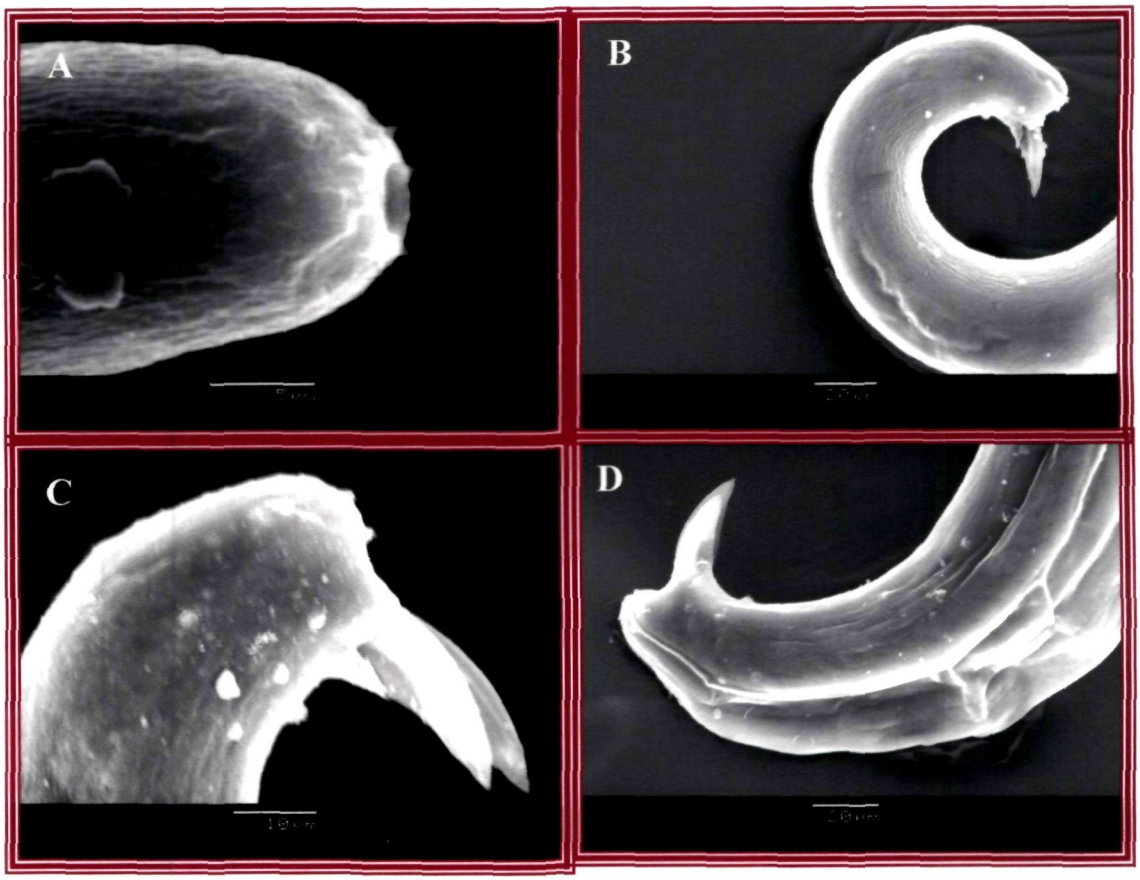


PLATE 1.16 *Steinernema thermophilum*
(Scanning electron micrographs)

Figs. A-D. First generation male; **A.** Anterior end showing mouth opening and labial papillae. **B.** Posterior region showing spicules and papillae **C.** Posterior region showing spicules and anal papillae **D.** Enlarged posterior region showing spicules and anal papillae

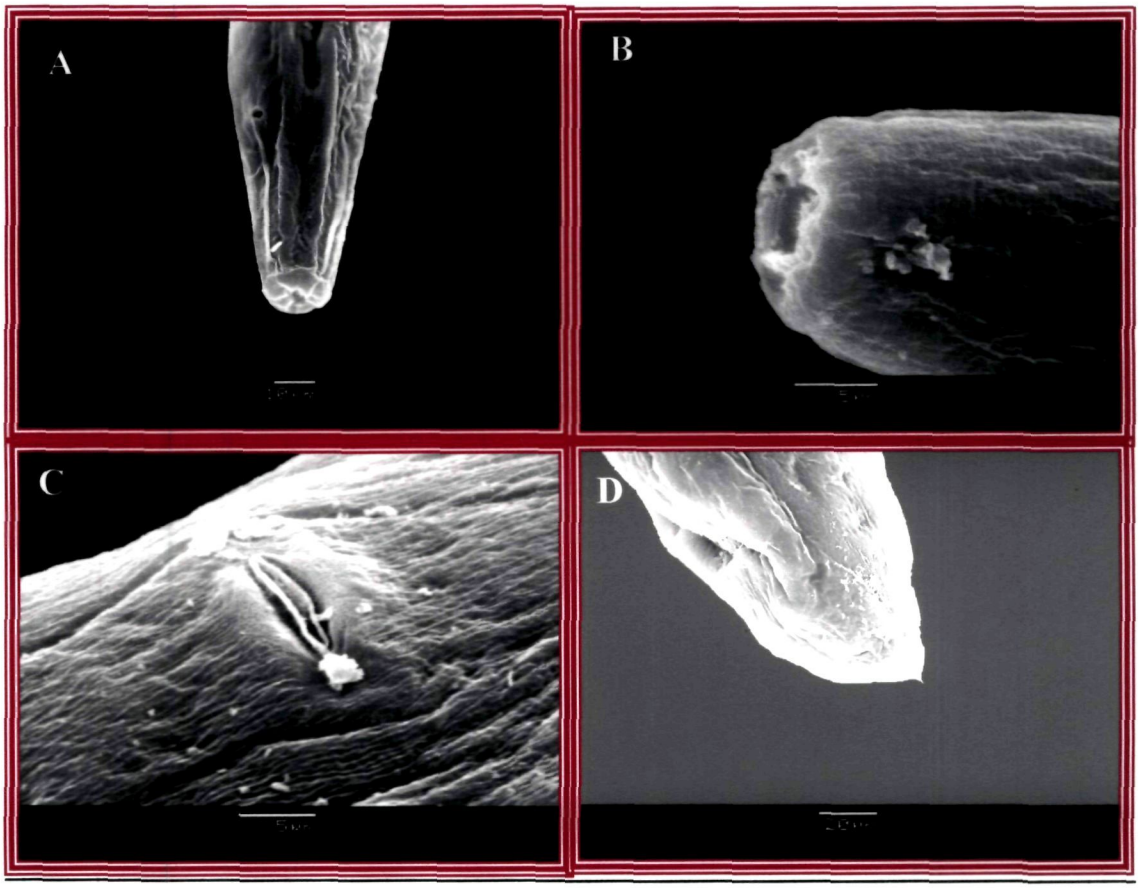


PLATE 1.17 *Steinernema thermophilum*
(Scanning electron micrographs)

Figs. A-D. Second generation female; **A.** Anterior region mouth opening **B.** Enlarged anterior region showing mouth opening and papillae **C.** Vulvar opening **D**. Posterior end showing mucronated tail tip.

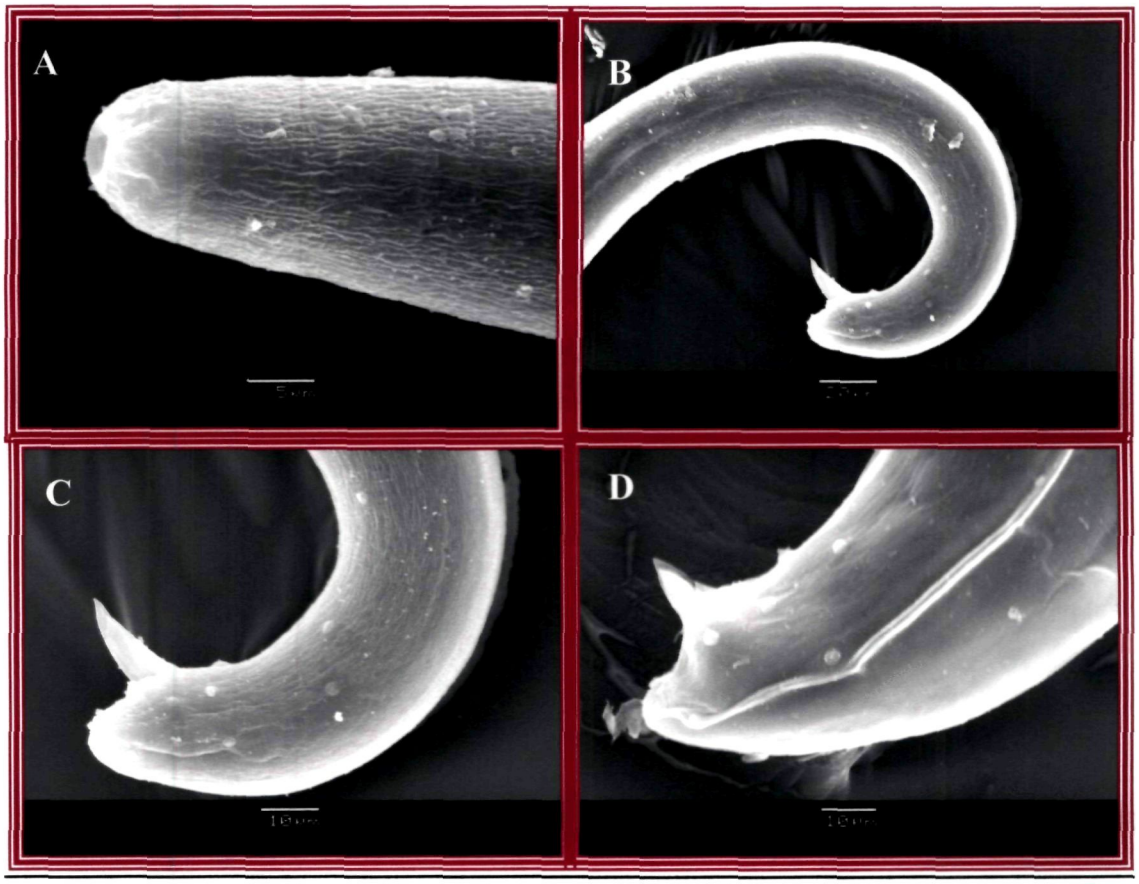


PLATE 1.18 *Steinernema thermophilum*
(Scanning electron micrographs)

Figs A-D. Second generation male; **A.** Anterior end showing mouth opening and labial papillae. **B.** Posterior region showing spicules and papillae **C.** Another view of posterior region showing spicules and anal papillae **D.** Enlarged posterior region showing spicules and anal papillae

mated females invariably with a jelly-like extrusion (mating plug) around their vulval aperture. Tail short, rounded with a small protuberance at terminus. In immature females, tail ventrally convex to form a cone with blunt, slightly dorsally directed terminus. Mucron at tail terminus absent.

Female, second generation:

Similar to the first generation female in most of the morphological details, except that they are smaller than first generation females. Tail elongate, acute conoid with a small spine-like mucron at terminus.

Male, first generation:

Body ventrally curved in the posterior region. Cuticle thin and smooth. Head almost truncated with distinct six labial and four cephalic papillae. Amphids pore-like located posterior to the lateral labial papillae. Stoma short and wide, with sclerotised cheilorhabdions. Oesophagus short in relation to body length. Procorpus almost cylindrical, metacorpus slightly swollen; isthmus present; basal bulb pyriform. Testis single and reflexed. Vas deferens with weak musculature. Spicules paired, arcuate with two internal ribs. Rostrum present; lamina tapering posteriorly. Distal tips of spicules sharply pointed. Gubemaculum flattened to bow shaped with proximal portion sharply pointed, straight or turned upward. Spicules and gubernaculum yellowish-brown in color. Tail with 25-27 genital papillae, with 11 pairs subventral, 2 pairs lateral and 1 single

prominent ventral preanal. Seven pairs subventral preanal located up to a distance of 350-400 μm anterior to cloacal aperture; 1 pair lateral preanal, 1 pair lateral postanal, 2 pairs subventral postanal, 1 pair subterminal and 1 pair adanal or absent sometimes. Tail short, bluntly conical with rounded terminus. Mucron at tail tip absent.

Male, second generation:

Similar to first generation males except for having smaller body size, smaller spicules and gubernaculum. Mucron at tail terminus absent.

Infective juveniles:

Body slender, tapering gradually to both ends. Cuticle with transverse striations. Lateral field distinct, marked by nine incisures (8 ridges). Lip region elevated, dome-shaped and set off from the body by constriction. Two horn-like structures present at the lip region. Stoma closed. Nerve ring just anterior to basal bulb. Basal bulb with a reduced valve. Excretory pore located at the level slightly anterior to the middle of esophagus. Hemizonid far posterior to excretory pore, at the level of nerve ring. Rectum long, slightly smaller than the anal body width. Tail elongate conoid, about 3-4 anal body widths long with acute terminus. Hyaline part of the tail covering less than half of the tail length.

The morphometric measurements of *S. thermophilum* are given in Tables 1.4 – 1.8. The comparisons of the morphometric data of S.

Table 1.13: Morphometric comparision of infective juveniles of *Steinernema thermophilum* with other related EPN species.

	S. thermophilum	*S. siamkayi*	*S. ritteri*	*S. rarum*	*S tami*	*S. abbasi*	*S. carpocapsae*	*S. riobrave*
BL	**542.75 ± 6.64** (513 - 658.3)	**446** (398 – 495)	**510** (470 – 590)	**511** (443 – 573)	**530** (400 – 600)	**541** (496 – 579)	**558** (438 – 650)	**622** (561 – 701)
BW	**23.77 ± 0.31** (22.80 - 28.50)	**21** (18 – 24)	**21.5** (19 – 24)	**22** (19 – 26)			**25** (20 – 30)	**28** (26 – 30)
ES	**98.04 ± 0.74** (91.20 - 102.60)	**94.5** (80 – 107)	**91.5** (85 – 95)	**102** (89 – 120)	**117** (110 – 123)	**89** (85 – 92)	**120** (103 – 190)	**114** (109 – 116)
EP	**44.12 ± 0.37** (39.90 - 45.60)	**35** (29 – 38)	**43** (40 – 46)	**38** (32 – 40)	**36** (34 – 41)	**48** (46 – 51)	**38** (30 – 60)	**56** (51 – 64)
ABW	**14.36 ± 0.20** (11.40 - 17.10)	**11.5** (9 – 15.5)	**14** (12 – 17)					**16** (15 – 17)
TL	**52.48 ± 0.95** (39.9 - 57)	**35.5** (31 – 41)	**49** (44 – 54)	**51** (44 – 56)	**50** (42 – 57)	**56** (52 – 61)	**53** (46 – 61)	**54** (46 – 59)
A	**22.86 ± 0.21** (20.00 - 25.25)	**21** (19 – 23)	**24.1** (19.5 – 30.9)				**21** (19 – 24)	**22.5** (20.1 – 23.5)
B	**5.54 ± 0.07** (5.11 - 6.42)	**4.7** (4 – 6.1)	**5.5** (4.9 – 6.3)				**4.4** (4.0 – 4.8)	**5.4** (4.9 – 6.0)
C	**10.42 ± 0.19** (9.25 - 12.93)	**11.3** (10.3 – 14.8)	**10.6** (9.2 – 13.1)	**9.8** (8.7 – 11)	**11** (9 – 12)	**9.8** (8.1 – 10.8)	**10** (9.1 – 11.2)	**11.6** (10.1 – 12.4)
D	**0.45 ± 0.01** (0.42 - 0.5)	**0.37** (0.31 – 0.43)	**0.46** (0.44 – 0.50)	**0.35** (0.30 – 0.39)	**0.31** (0.28 – 0.34)	**0.53** (0.51 – 0.58)	**0.26** (0.23 – 0.28)	**0.49** (0.45 – 0.55)
E	**0.85 ± 0.02** (0.70 - 1.07)	**0.96** (0.85 – 1.12)	**0.88** (0.79 – 0.97)	**0.72** (0.63 – 0.80)	**0.73** (0.67 – 0.86)	**0.86** (0.79 – 0.94)	**0.60** (0.54 – 0.66)	**1.05** (0.93 – 1.11)
F	**0.46 ± 0.01** (0.40 - 0.57)							

Table 1.14: Morphometric comparision of 1st generation female of *Steinernema thermophilum* with other related EPN species.

	S. thermophilum	*S. siamkayi*	*S. ritteri*	*S. rarum*	*S. riobrave*
BL	**4190.8 ± 185.11** (3063.75 to 6099)	**3937** (3161 –5172)	**8600** (5200 – 11500)	**7390** (5200 – 10400)	**6500** (3700 – 8300)
BW	**170.86 ± 2.96** (148.20 to 210.90)	**198** (170 – 280)	**252.2** (210 – 300)	**169** (132 – 216)	**275** (200 – 390)
StL	**5.70 ± 0.00** (5.70 to 5.70)	**8** (6 – 10)	**9.5** (7 – 13)	**9** (6.5 – 12)	**5.7** (4.3 – 6.3)
StW	**10.45 ± 0.30** (8.55 to 11.40)	**9.5** (7.5 – 12)	**9** (6 – 12)	**11** (9 – 12.5)	**8** (7.1 – 8.8)
ES	**168.15 ± 2.90** (151.05 to 216.6)	**177** (152 – 199)	**196** (130 – 232)	**219** (205 – 250)	**193** (171 – 211)
EP	**83.46 ± 2.34** (62.70 to 99.75)	**66** (50 – 84)	**74.5** (40 – 135)	**93** (65 – 113)	**96** (80 – 118)
ABW	**62.29 ± 0.99** (51.30 to 71.25)	**51** (48 – 53)	**66** (45 – 95)	**84** (65 – 110)	**93** (63 – 115)
TL	**30.81 ± 0.51** (25.65 to 34.20)	**31** (22 – 37.5)	**27.6** (19 – 36)	**44** (37 – 50)	**45** (41 – 50)
D	**0.51 ± 0.01** (0.39 to 0.61)				**0.49** (0.42 – 0.62)
E	**2.74 ± 0.09** (1.83 to 3.50)				
V%	**52.43 ± 0.63** (47.79 to 57.64)	**51** (48 – 53)	**51** (44 – 56)	**57** (52 – 66)	**52** (49 – 56)

Table 1.15: Morphometric comparision of 1st generation male of *Steinernema thermophilum* with other related EPN species.

	S. thermophilum	*S. siamkayi*	*S. ritteri*	*S. rarum*	*S. tami*	*S. abbasi*	*S. carpocapsae*	*S. riobrave*
BL	**1057.92 ± 27.69** (983.25 to 1140)	**1135** (1035 – 1278)	**1480** (1200 – 1900)	**1600** (1300 – 1700)			**1450** (1090 – 1710)	**1700** (1500 – 1900)
BW	**88.35 ± 1.81** (85.50 to 94.05)	**139.5** (107 – 159)	**130** (110 – 176)	**123** (100 – 142)			**102** (77 – 131)	**133** (116 – 160)
StL		**3.5** (3 – 4)	**2.9** (1.5 – 5)	**3** (2 – 5)				
StW		**4** (3 – 5)	**4.40** (3.5 – 5)	**4.5** (3 – 5)				
ES	**128.25 ± 2.89** (116.80 to 133.90)	**134** (123 – 141)	**137** (121 – 155)	**135** (125 – 150)			**155** (136 – 167)	**144** (128 – 154)
EP	**76.95 ± 0.91** (74.10 to 79.80)	**57** (47.5 – 67)	**65.5** (53 – 78)	**67** (55 – 77)			**61** (47 – 74)	**103** (94 – 111)
NR	**88.35 ± 3.04** (85.50 to 96.90)		**105** (92.5 – 124)	**103** (91 – 120)			**110** (93 – 124)	**115** (106 – 134)
ABW	**37.62 ± 0.57** (37.05 to 39.90)	**45** (47 – 65)	**44** (37 – 52)	**50** (40 – 57)			**42.6** (32.5 – 54.6)	**59** (50 – 64)
TL	**28.50 ± 0.91** (25.65 to 31.35)	**27.5** (22 – 32)	**26.5** (21 – 32)	**43** (35 – 52)			**30** (23.4 – 39)	**31** (29 – 35)
SPL	**62.7 ± 0.91** (59.85 to 65.55)	**77.5** (75 – 80)	**69** (58 – 75)	**48** (42 – 52)	**72.77** (63 – 78)	**65** (57 – 74)	**64.6** (58.5 – 71.5)	**67** (63 – 75)
GL	**31.35 ± 0.02** (31.35 to 34.20)	**53.5** (47 – 65)	**43.5** (33 – 50)	**34** (23 – 38)		**45** (33 – 50)	**47** (39 – 56)	**51** (47.5 – 56.2)
D	**0.61 ± 0.02** (0.57 to 0.68)	**0.42** (0.35 – 0.49)	**0.47** (0.44 – 0.50)	**0.50** (0.44 – 0.51)	**0.44** (0.30 – 0.60)	**0.60** (0.51 – 0.68)	**0.41** (0.27 – 0.55)	**0.71** (0.60 – 0.80)
E	**2.71 ± 0.11** (2.45 to 3.00)	**2.07** (1.66 – 2.57)						
SW	**1.67 ± 0.03** (1.57 to 1.77)	**1.7** (1.40 – 2.20)	**1.6** (1.40 – 1.60)	**0.90** (0.90 – 1.05)	**2.0** (1.40 – 3.0)	**1.56** (1.07 – 1.87)	**1.7** (1.40 – 2.00)	**1.1**

thermophilum with other related species in the genus are provided in Tables 1.13 – 1.15.

Steinernema glaseri

Class	Secernentea von Linstow, 1905
Order	Rhabditida (Orley, 1880) Chitwood, 1933
Suborder	Rhabditina (Orley, 1880) Chitwood, 1933
Superfamily	Rhabditoidea (Orley, 1880) Travassos, 1920
Family	Steinernematidae Chitwood and Chitwood, 1937
Genus	*Steinernema* Travassos, 1927 *S. glaseri* (Steiner 1929) Wouts, Mracek, Gerdin and Bedding, 1982 (Syn. *N. glaseri* Steiner, 1929)

(Plates: 1.19 – 1.28)

Females:

Cuticle smooth, head slightly rounded. Six distinct lips united, each with 1 papilla. Four cephalic papillae present. Amphids crescent-shaped, narrow. Stoma partially collapsed. Oesophagus muscular with a cylindrical procorpus followed by a slightly swollen non-valvated metacorpus, isthmus, and basal bulb with a valve. Nerve ring surrounding the isthmus just anterior to tile basal bulb. Excretory pore opening anterior to nerve ring. Lateral fields and phasmids inconspicuous. Gonads amphidelphic, reflexed. Vulva in the form of a transverse slit, protruding from the body surface, with a thick flap.

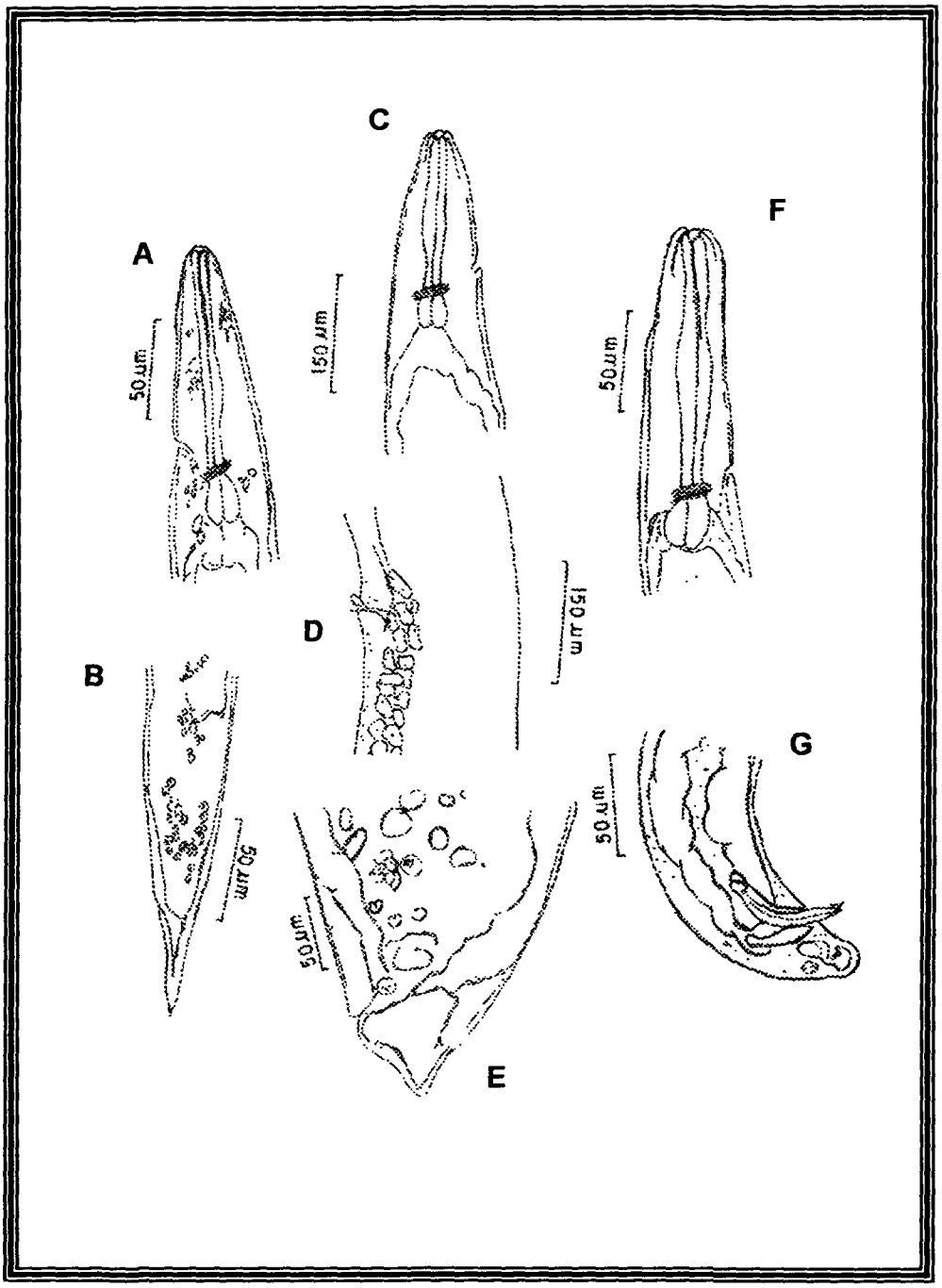


PLATE 1.19 *Steinernema glaseri*

Figs. A-B. Infective juvenile; **A.** Anterior end showing excretory pore, oesophagous and nerve ring **B.** Posterior end showing tail tip. **C-E First generation female**; **C.** Anterior end showing excretory pore, oesophagous and nerve ring **D.** Vulvar opening **E.** Posterior end showing anal pore **Figs. F-G. First generation male; F.** Anterior end showing excretory pore, oesophagous and nerve ring **G.** Posterior end showing spicule and gubernaculum.

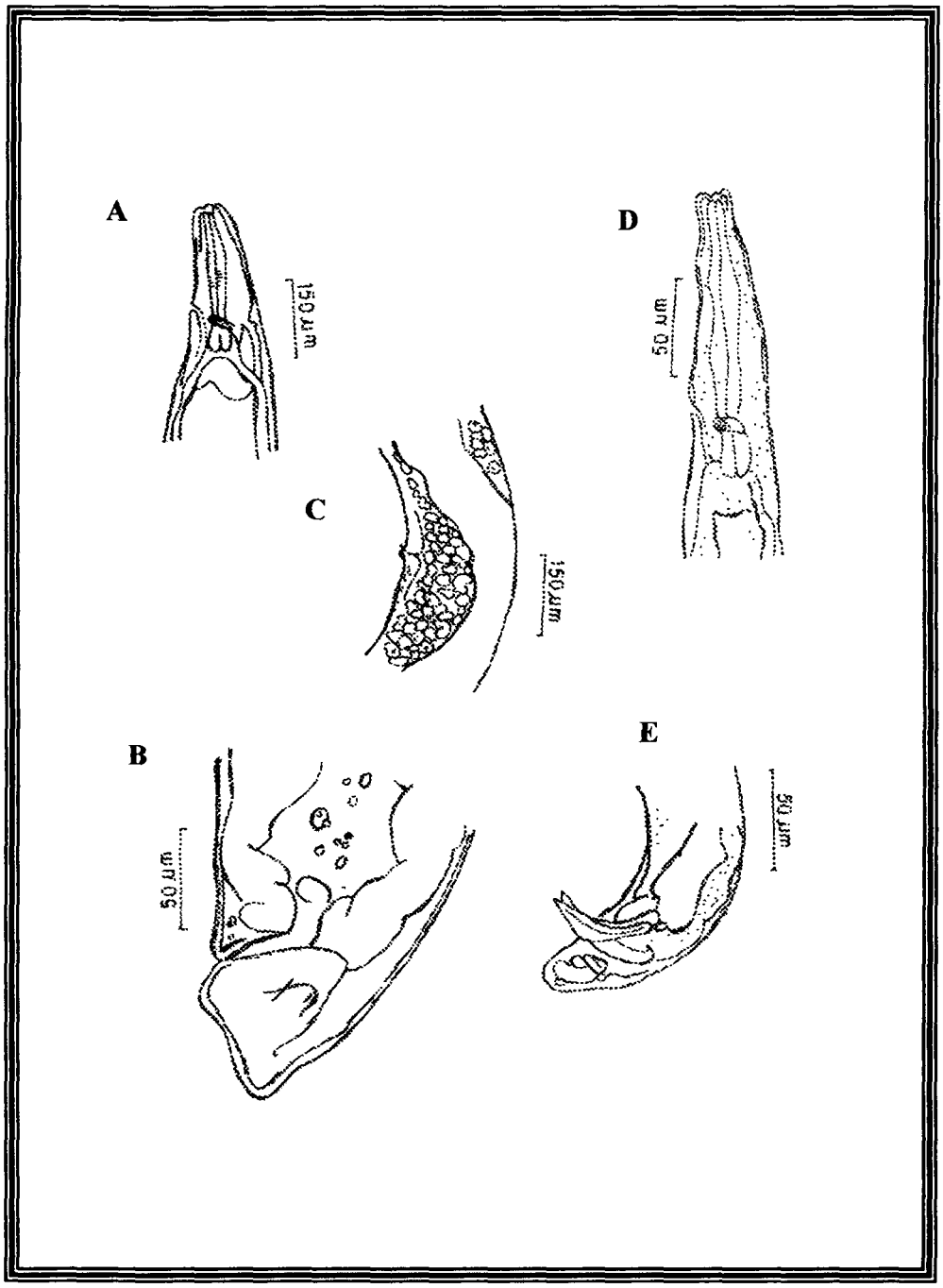


PLATE 1.20 *Steinernema glaseri*

Figs. A-C. Second generation female; **A.** Anterior end showing excretory pore, oesophagous and nerve ring **B.** Posterior end showing anus and tail tip **C.** Vulvar opening **Figs. D-E. Second generation male**; **D.** Anterior end showing excretory pore oesophagous and nerve ring **E.** Posterior end showing spicules and gubernaculum.

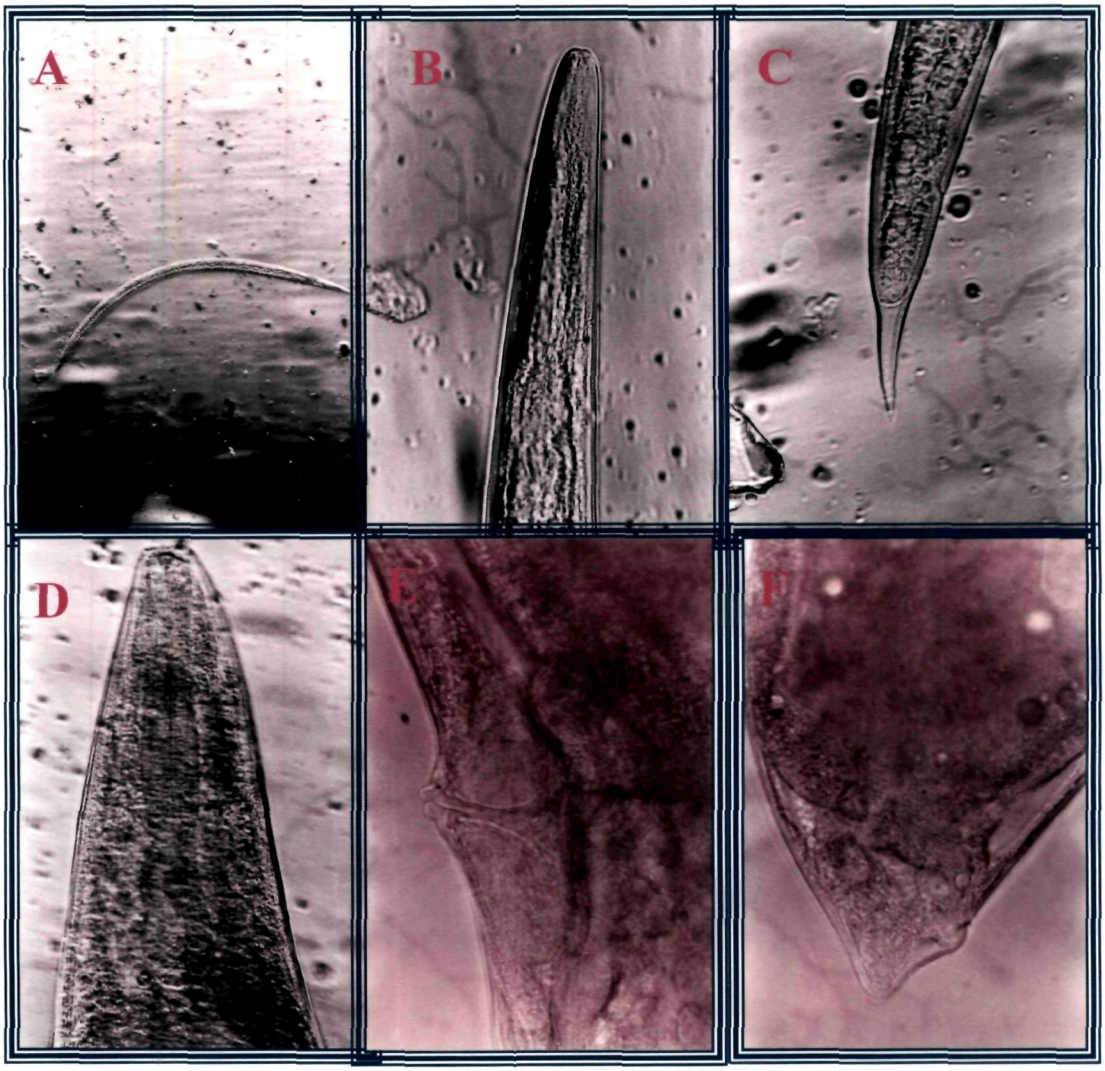


PLATE 1.21 *Steinernema glaseri* (Photomicrographs)

Figs. A-C. Infective juvenile; **A.** Whole body* **B.** Anterior end showing oesophagus **C.** Posterior end showing pointed tail tip **Figs. D-F. First generation female**; **D.** Anterior end showing oesophagus and excretory pore and oesophagus **E.** Vulvar opening **F.** Posterior end showing anal region.

(* *4X*, ** *25X, all other photographs are taken at 40X*)

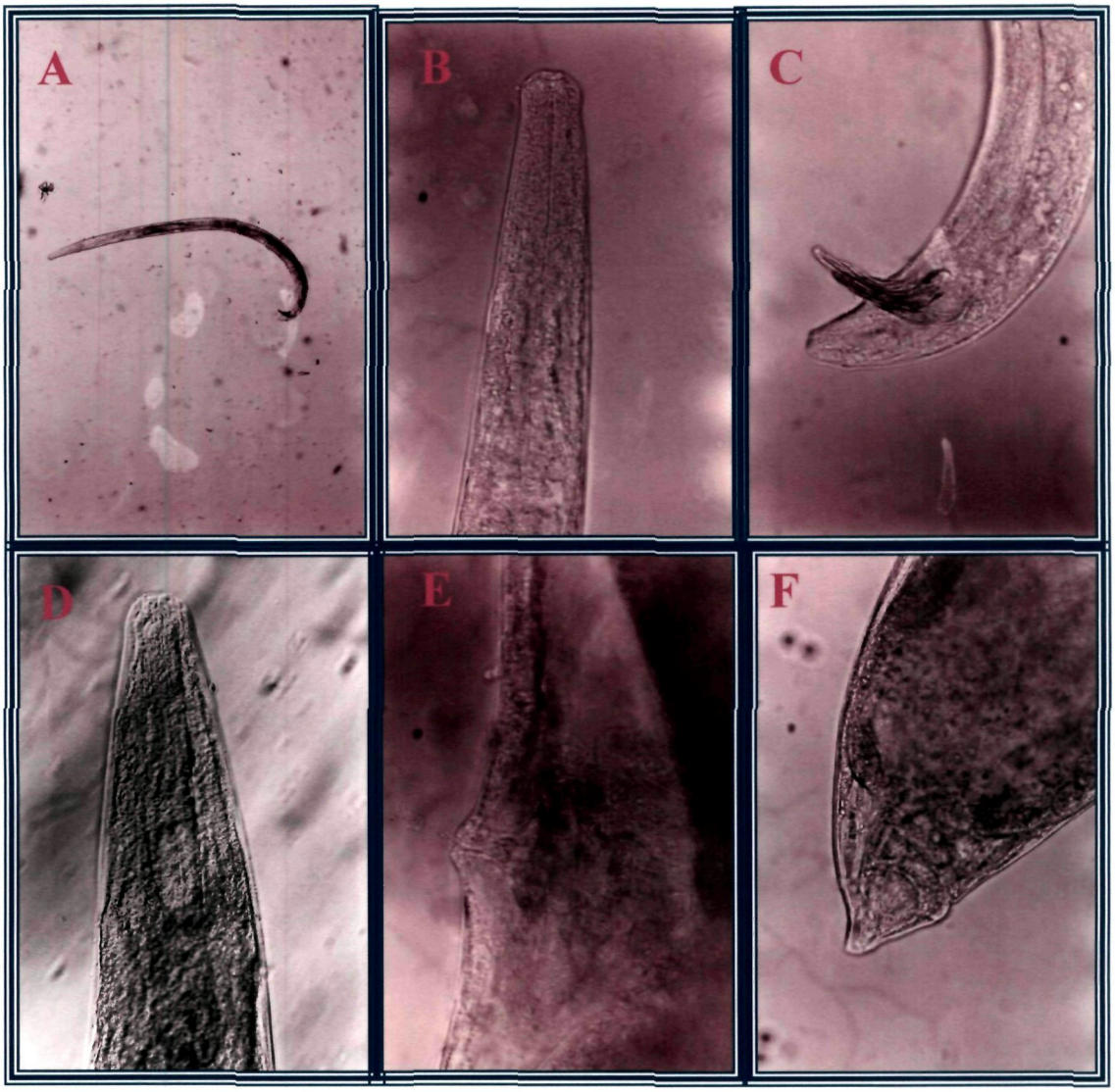


PLATE 1.22 *Steinernema glaseri* (Photomicrographs)

Figs. A-C. First generation male; **A.** Whole body* **B**. Anterior end showing oesophagus **C**. Posterior end showing spicules and gubernaculum **Figs. D-F. Second generation female**; **D.** Anterior end showing oesophagus **E.** Vulvar opening **F.** Posterior end showing anal region.

(4X, ** 25X, all other photographs are taken at 40X)*

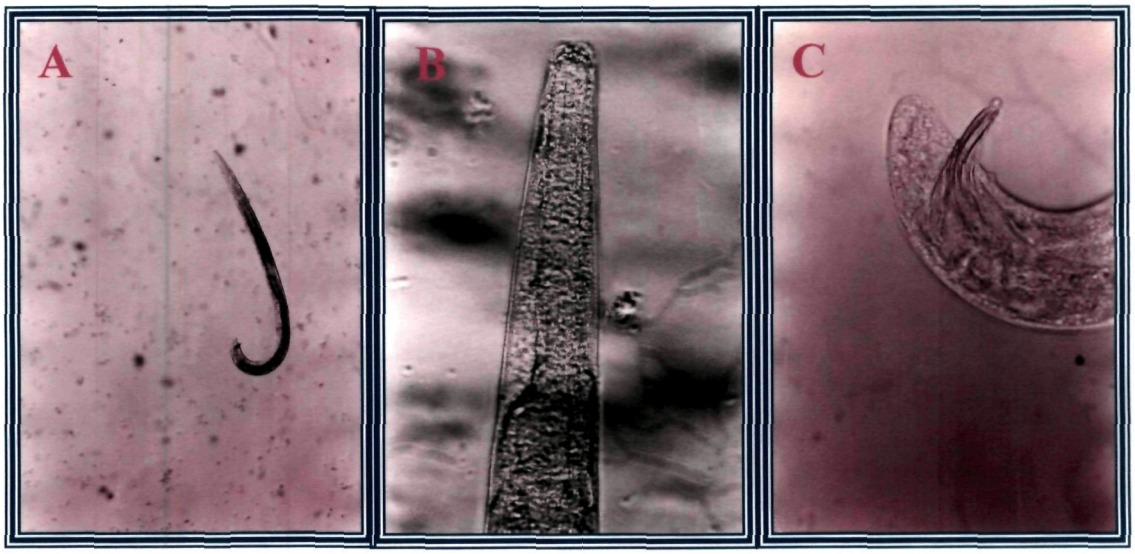


PLATE 1.23 *Steinernema glaseri* (Photomicrographs)

Figs. A-C. Second generation male; **A**. Whole body* **B**. Anterior end showing oesophagus **C**. Posterior end showing spicules and gubernaculums.

(4X, all other photographs are taken at 40X)*

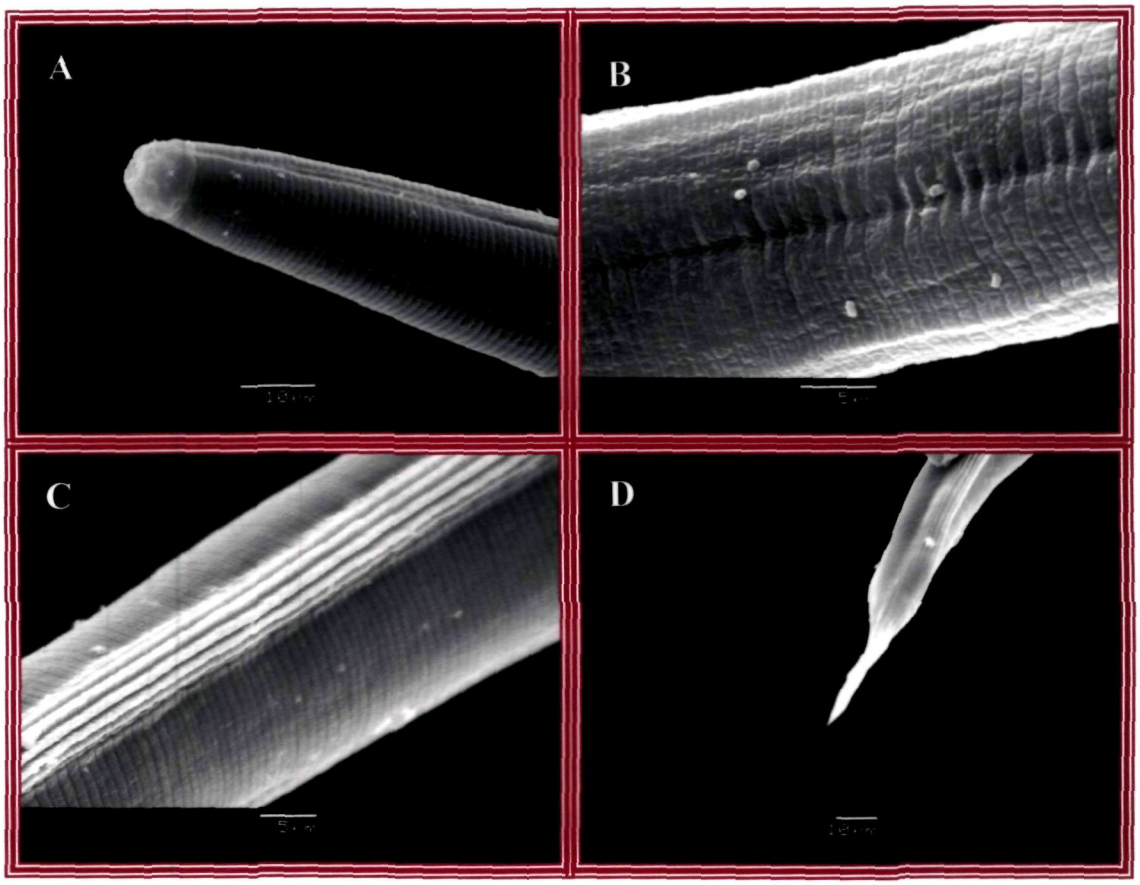
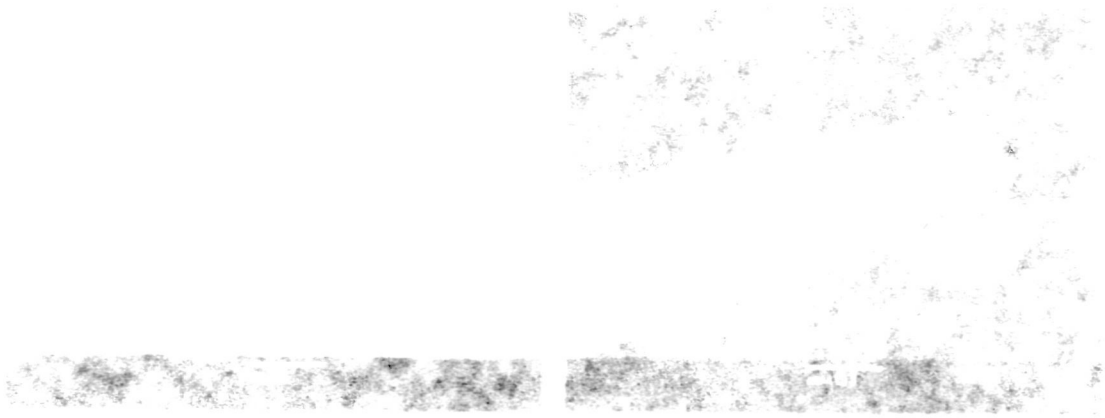


PLATE 1.24 *Steinernema glaseri*
(Scanning electron micrographs)

Figs. A-D. Infective juveniles; **A.** Anterior region showing transverse cuticular striations **B.** Ventral ridges **C**. Longitudinal ridges **D.** Posterior end showing tail tip.



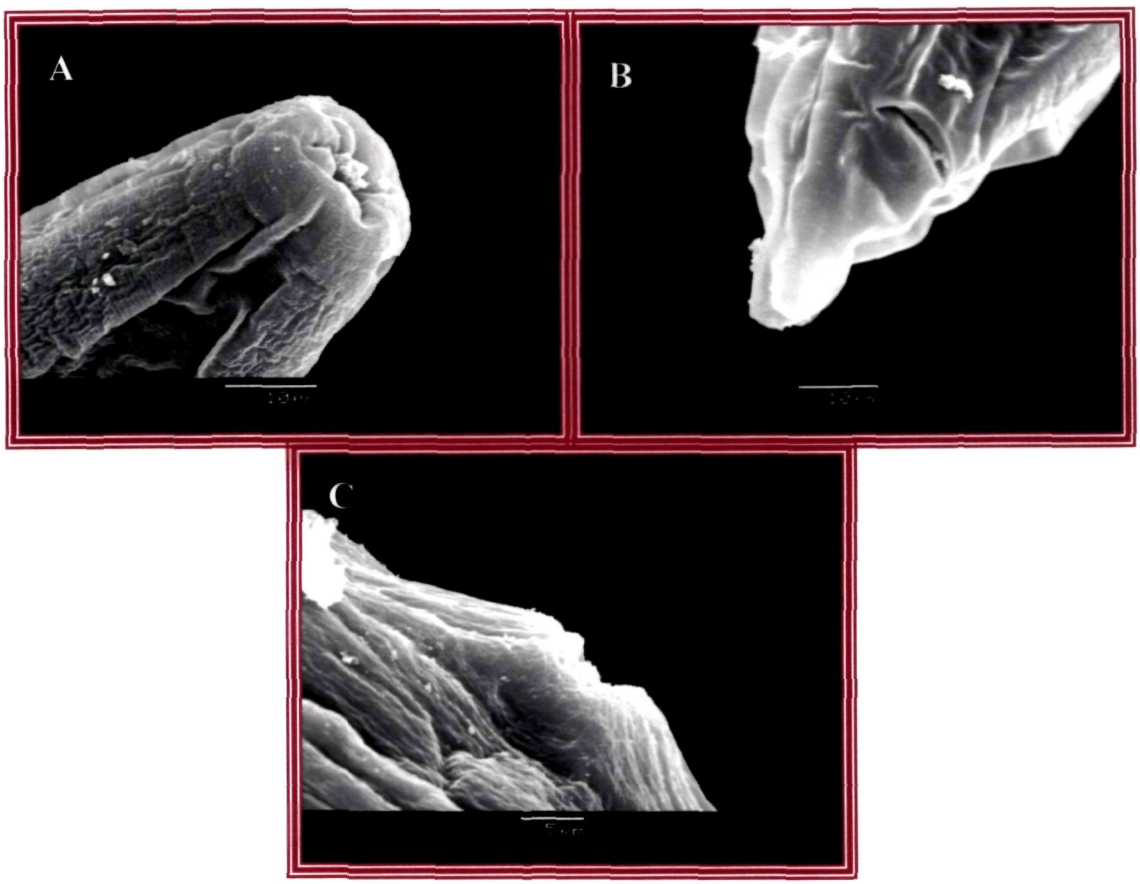


PLATE 1.25 *Steinernema glaseri*
(Scanning electron micrographs)

Figs. A-C. First generation female; **A**. Anterior end showing lip region **B.** Posterior end showing anal opening **C.** Vulvar opening.

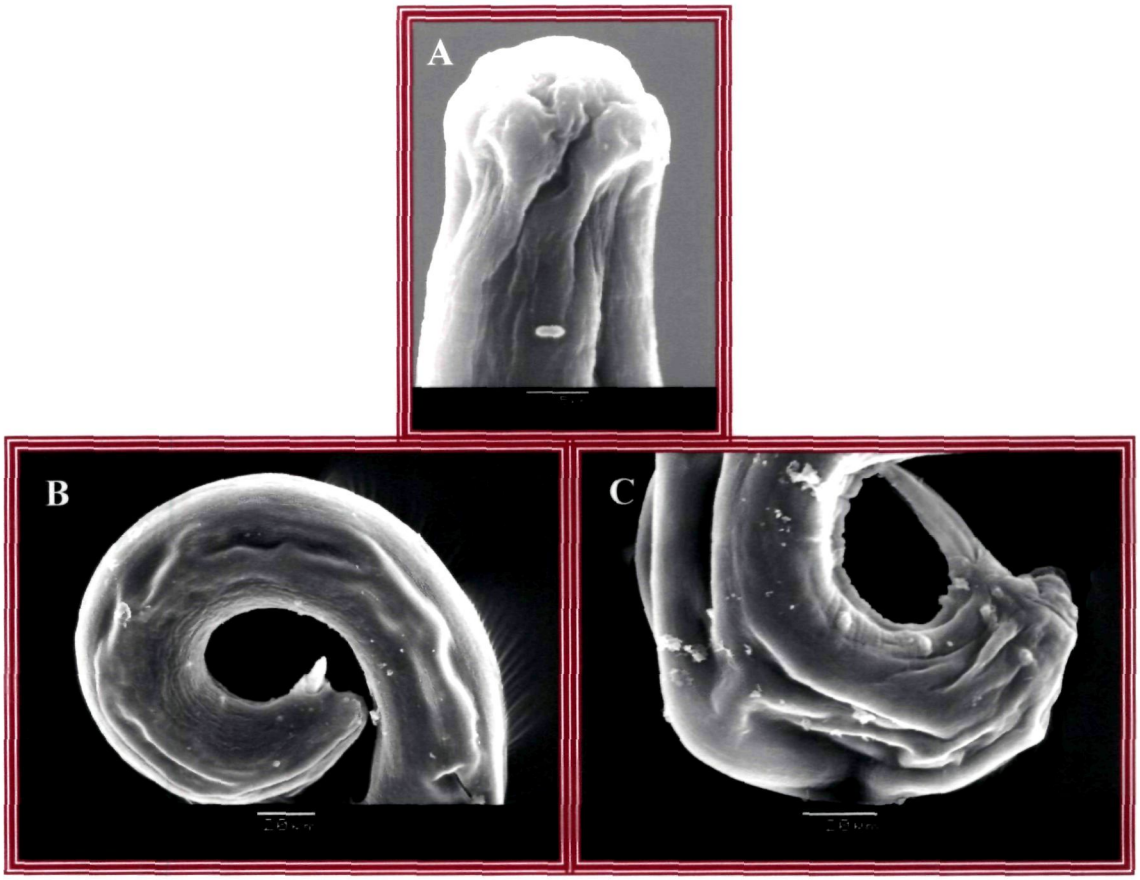


PLATE 1.26 *Steinernema glaseri*
(Scanning electron micrographs)

Figs. A-C. First generation male; **A**. Anterior end **B**. Posterior region showing spicules and papillae **C**. Posterior region showing spicules.

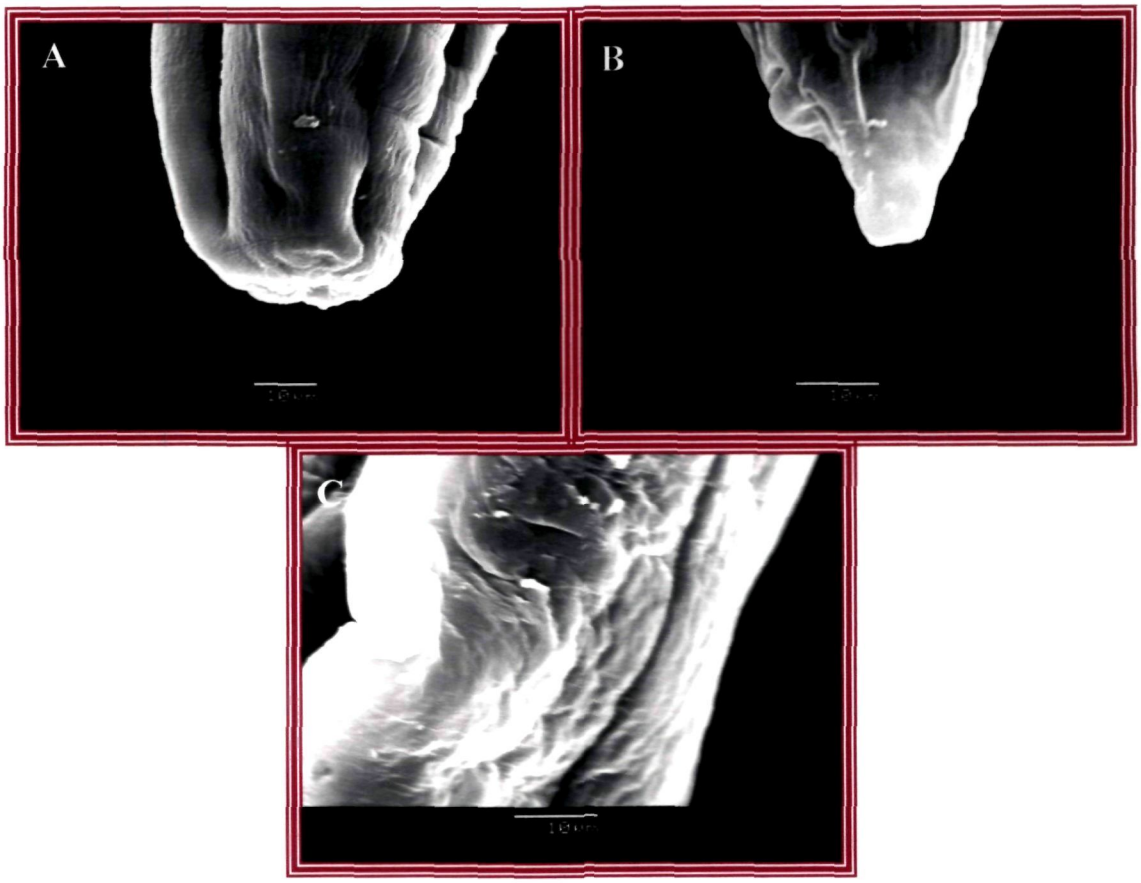


PLATE 1.27 *Steinernema glaseri*
(Scanning electron micrographs)

Figs. A-C. Second generation female; **A.** Anterior end **B.** Posterior region showing anal opening and tail. **C.** Vulvar opening.

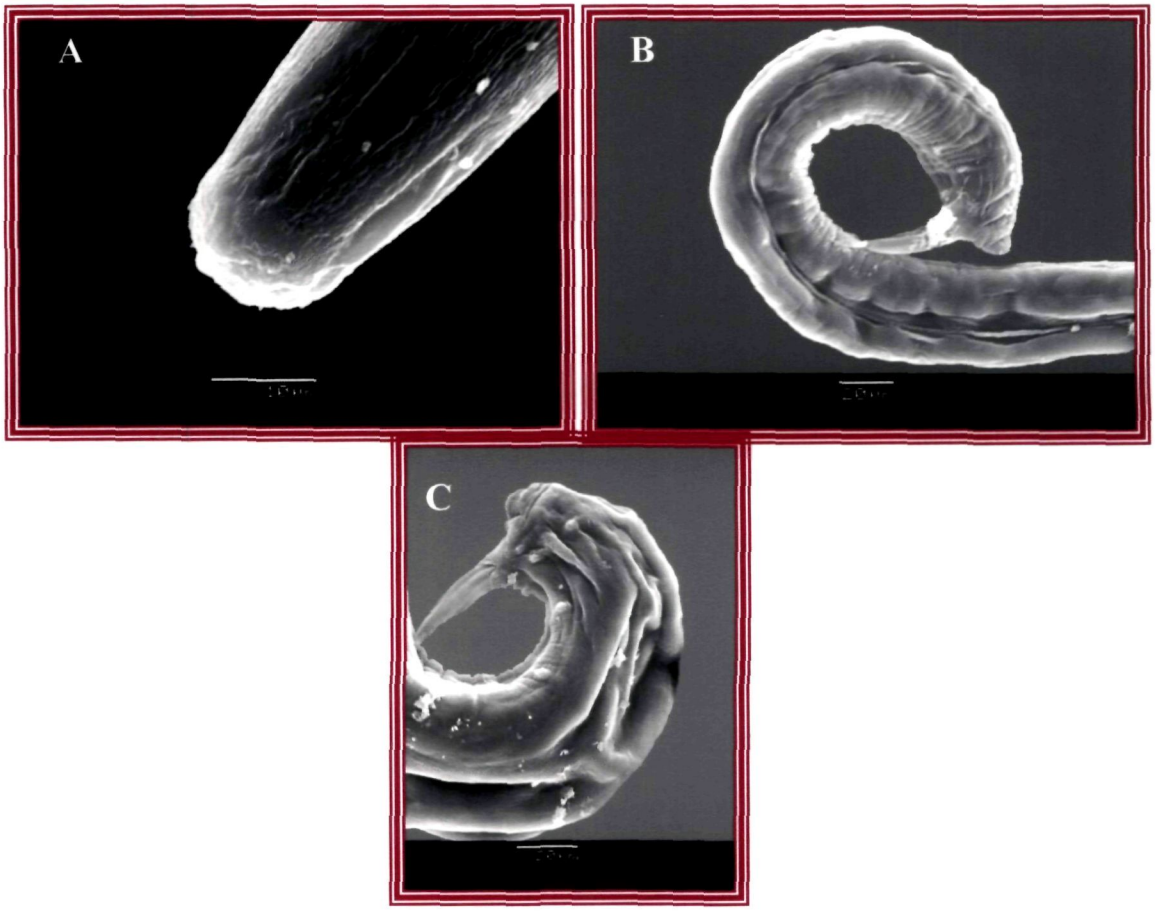


PLATE 1.28 *Steinernema glaseri*
(Scanning electron micrographs)

Figs. A-C. Second generation male; **A.** Anterior region **B**. Posterior region showing spicules **C**. Enlarged posterior end showing spicules and papillae.

Vagina short leading into paired uteri filled with eggs and juveniles. First generation females larger than those of the second generation. Tail with a prominent postanal swelling, terminating with a rounded projection in the first generation females.

Males:

General morphology, same as the females. With a single reflexed testis. Spicule head short, shaft distinct, head comprises about 24% (21-25%) of the spicule length. Blade long and narrow bearing two ridges. Distal tip of spicules bearing a ventral aperture which make it appear "hook- or "notch-like. Velum absent. Gubernaculum variable in shape, anterior end curved ventrally, enlarged gradually posteriorly. Two wings of corpus curved upward, usually capitulum and cuneus forked anteriorly to form a Y-shape. Tail with twenty-three genital papillae (eleven pairs and a single ventral preanal). Five of the 11 pairs preanal, subventral, pair six lateral, pairs seven and eight subventral adanal, pairs nine and ten subventral and subterminal, pair eleven subdorsal.

Infective juveniles:

Head without annules, labial papillae not observed, four cephalic papillae and amphids pronounced. Mouth and anus closed, and esophagus and intestine collapsed. Tail conoid and slightly straight; hyaline portion is less than 1/2 of the tail length. Lateral field pattern begins anteriorly with one line at third annule, two lateral lines appear at annules 3-5 to form two ridges. At the level of the isthmus and basal

bulb, the number of ridges in the lateral field increases from two to five and the lateral field is areolated. A short distance posteriorly, the central ridge divides into two making six ridges in the lateral field, and areolation becomes less obvious. More posteriorly, two additional lines appear on either side of the lateral field to form two new ridges, making eight ridges, the maximum in the lateral field. Near the level of anus, the four central ridges enlarged. Phasmids located at almost the same level near mid-tail with a pore at the centre. No annules observed posterior to the phasmids on the ventral surface and only a few were observed on the dorsal surface.

The morphometric measurements of *S. glaseri* are given in Tables 1.4 – 1.8. The comparisons of the morphometric data of *S. glaseri* with other related species in the genus are provided in Tables 1.16 – 1.17.

Table 1.16: Morphometric comparision of infective juveniles of *Steinernema glaseri* with other related EPN species.

	S. glaseri	*S. oregonense*	*S. loci*	*S. diaprepesi*	*S. arenarium*	*S. longicaudatum*	*S. puertoricense*	*S. cubanum*
BL	**1155.28 ± 19.95** (974.45 - 1336.1)	**980** 820 - 1110	**986** 896 – 1072	**1002** 880 – 1133	**1034** 724 – 1408	**1063**	**1171** 1057 – 1238	**1283** 1149 – 1508
BW	**45.37 ± 0.79** (39.90 - 54.15)	**34** 28 – 38						
ES	**157.21 ± 0.88** (148.20 - 165.30)	**132** 116 – 148	**141** 126 – 155	**138** 111 – 152	**138** 123 – 160	**145**	**143** 138 – 147	**148** 135 – 159
EP	**104.88 ± 1.14** (85.50 - 116.85)	**66** 60 – 72	**80** 71 – 86	**74** 66 – 83	**83** 76 – 86	**81**	**95** 90 – 102	106 101 – 114
ABW	**29.98 ± 0.29** (28.50 - 31.35)							
TL	**89.38 ± 1.65** (68.40 - 102.60)	**70** 64 – 78	**75** 66 – 83	**83** 65 – 91	**75** 64 – 84	95	**94** 88 – 107	**67** 61 – 77
A	**30.68 ± 0.49** (23.56 - 34.87)	**30** 24 – 37						
B	**8.82 ± 0.12** (6.98 - 9.8)	**7.6** 6 – 8						
C	**15.62 ± 0.34** (12.86 – 20.00)	**14** 12 – 16	**13** 11 – 15	**12** 10.4 – 13.2	**13.8** 9.4 – 16.9	**11.2**	**12.4** 11.6 – 13.6	**19.2**
D	**0.67 ± 0.01** (0.56 - 0.71)	**0.50** 0.40 – 0.60	**0.57** 0.52 – 0.63	**0.54** 0.30 – 0.70	**0.55** 0.52 – 0.59	**0.56**	**0.66** 0.62 – 0.74	**0.70**
E	**1.19 ± 0.02** (1.03 - 1.54)	**1** 0.90 – 1.10	**1.07** 0.94 – 1.20	**0.90** 0.78 – 1.14	**1.19** 1.06 – 1.30	**0.85**	**1.01** 88 – 108	**1.60**
F	**0.51 ± 0.01** (0.40 - 0.66)							

Table 1.17: Morphometric comparision of 1st generation male of *Steinernema glaseri* with other related EPN species.

	S. glaseri	*S. oregonense*	*S. loci*	*S. diaprepesi*	*S. arenarium*	*S. longicaudatum*	*S. puertoricense*	*S. cubanum*
BL	**1413.32 ± 31.26** (1291.05 - 1573.20)							
BW	**69.26 ± 2.95** (48.45 - 82.65)							
ES	**165.30 ± 5.09** (136.8 - 190.95)							
EP	**117.99 ± 5.41** (91.20 - 145.35)							
ABW	**42.18 ± 1.19** (34.20 - 48.45)							
TL	**37.62 ± 1.02** (34.20 - 42.75)							
SPL	**73.53 ± 3.15** (59.85 - 94.05)	**71** 65 – 73	**71** 60 – 80	**79** 71 – 90	**84** 81 – 91	**77**	**78** 71 – 88	**58** 50 – 67
GL	**44.18 ± 1.14** (39.90 - 48.45)	**56** 51.5 – 59	**46** 40 – 52	**54** 45 – 61	**55** 49 – 60	**48**	**40** 36 – 45	**39** 37 – 42
D	**0.71 ± 0.02** (0.60 - 0.81)	**0.73** 0.64 – 0.75	**0.73** 0.61 – 0.80	**0.80** 0.68 – 0.86	**0.93** 0.88 – 1.02	**0.62**	**0.77**	**0.70**
E	**3.16 ± 0.18** (2.40 - 4.25)							
SW	**1.75 ± 0.07** (1.40 - 2.20)	**1.51**	**1.9** 1.7 – 2.1	**1.8** 1.5 – 2.0	**2.1**	**1.6**	**1.5**	**1.4**

Discussion

One of the major constraints in agriculture production in India is the economic loss sustained due to attack by pest and diseases. Of these, the insect pests alone are considered to be more economically important, as one-third of the damage to the crops is estimated to be caused by them besides mites (Desbach and Rosen, 1991). Of various methods used for their control, the extensive use of chemical pesticides in particular has not only resulted in widespread insect resistance but other adverse effects on environment and human health as well. Other effective and environment friendly methods, therefore, are being explored and this trend has gained momentum during the recent years.

Nematodes associated with insects, referred to as entomophilic, entomogenous or entomopathogenic are known to parasitize, cause disease and kill the insects. Although 40 nematode families have been found associated with insects, only two families, Heterorhabditae and Steinernematidae have been widely used as biological control agents in agriculture system (Gaugler and Kaya, 1990; Kaya, 1990). The long viable infective juveniles (IJs) of nematodes under these families have the potential for long term establishment in soil through recycling on infected insects. Other attributes include compatibility with other control strategies including pesticides, ease of production, formulation and application (Georgis and Hom, 1992). Presently EPNs account for

most of biopesticides marketed in the industrialized countries, apart from *Bacillus thuringiensis* (Bedding, 1996).

The aim of the present study was to investigate the faunal composition and natural distribution of entomopathogenic nematodes in Meghalaya, a north-eastern state of India. For making the EPN survey, district Ri-Bhoi was selected as study area, since it is reported to have ecologically diverse type of soil habitats. The survey was conducted for a period of one year, using *G. mellonella* as baiting agent.

In the present study, out of 1656 soil samples collected from ecologically diverse type of habitats, 89 (5.37%) were found to be positive for EPNs. Further, the study revealed a pre-dominance of steinernematids (73.03%) over heterorhabditids (26.97%) in EPN positive soil samples. Rosa *et al.* (2000) have summarized the rate of recovery of EPNs from various soil surveys conducted throughout the world. Most surveys show their recovery rate from soils between 6 and 35% (Rosa *et al.*, 2000). Other surveys with 5% or less recovery of EPNs include, 2% in Turkey by Hazir *et al.* (2003), 2.20% in Scotland by Boag *et al.* (1992), 3.8% in Northern Ireland by Blackshaw (1988), 3.9% in the Azorean archipelago, Portugal by Rosa *et al.* (2000), 4.6% in Korea by Choo *et al.* (1995), 4.7% in Turkey by Ozer *et al.* (1995) and 5% in Italy by Ehlers *et al.* (1991). In other EPNs surveys, the recovery rates of EPNs have been reported to be between 8 and 30% (Midituri *et al.*, 1997; Griffin *et al.*, 1999; 2000).

Moderately higher prevalence rate of EPNs have been reported by Griffin *et al.* (1991) in Ireland (10.50%), Akhurst and Bedding (1986) in

Australia (11.00%), Midituri *et al.* (1996a) in West Flanders, Belgium (12.30%), Stock (1995) in Argentina (13.20%), Akhurst and Brooks (1984) in North California (20.00%), Mracek and Webstar (1993) in Western Canada (20.00%), Burman *et al.* (1986) in Sweden (25.00%), Steiner (1994) in Swiss Alps (26.50%), Rueda *et al.* (1993) in Tennessee (27.00%) and Mracek (1980) in Czechoslovakia (36.80%). Stock *et al.* (1999), while studying the distribution of EPNs in natural habitat in California, reported occurrence of EPNs in 71 (26.3%) of the 270 soil sample collected from 12 distinct habitats. Of these, 80% constituted the steinernematids and 20% heterorhabditids. In a recent study, Mracek *et al.* (2005) recorded their prevalence to be as high as 50.60% in Czech Republic.

Hazir *et al.* (2003b) reported that out of the 22 positive samples, 15 steinernematids (68.2%) and 7 heterorhabditids (31.8%) isolates were recovered from six of the seven regions in Turkey. Similarly, Uribe-Lorio *et al.* (2005) reported that EPNs were recovered from 5 out of the 41 samples collected, and among the positive samples, 4 out of 5 samples contained steinernematids, while only 1 sample contained heterorhabditid in Costa Rica. In the present study, the steinernematids were found to be significantly predominating than heterorhabditids. Many other workers have also reported dominance of steinernematids recovery over heterorhabditids. (Burman *et al.*, 1986; Garcia del Pino, 1996; Griffin *et al.*, 1991; Hominick *et al.*, 1995; Steiner, 1996; Sturhan and Liskova, 1999; Yoshida *et al.*, 1998). In contrast, the dominance of heterorhabditids over steinernematids has

been found in rather few surveys. For example, Rosa *et al.* (2000) in a study in nine islands of the Azorean archipelago noticed that *Heterorhabditis* spp. were present on 30 sites from six islands, whereas *Steinernema* spp. were found only on 16 sites from three islands. Similarly, Griffin *et al.* (1994) also reported a dominance of heterorhabditids over steinernematids in Britain and Ireland. Predominance of heterorhabditids over steinernematids has also been reported by Hara *et al.* (1991) in Hawaiian Islands and by Roman and Figueroa (1995) in Puerto Rico.

In India, studies by Raj Kumar *et al.* (2001) showed that out of 105 soil samples collected from Rajasthan, only 5 (4.76%) were found to be positive for steinernematids and heterorhabditids. Subsequently, Parihar *et al.* (2002) undertook another survey in Rajasthan and reported the presence of *Steinernema* sp. and *Heterorhabditis* sp. from 8 (1.68%) samples out of 477 samples studied. They further mentioned that out of 8 positive samples, 5 (62.5%) were positive for *Heterorhabditis* sp. and the other 3 (37.5%) constituted *Steinernema* sp. Josephrajkumar and Sivakumar (1997) in their study in Tamil Nadu reported the prevalence of steinernematids to be (94.44%) and of heterorhabditids (5.55%). In contrast to this, Singh *et al.* (1992) reported a very low prevalence (1.82%) of *Steinernema* sp. at ICRISAT centre, Hyderabad. Kaushal *et al.* (2000) examined 207 soil samples from diverse areas of India (Uttar Pradesh, Himachal Pradesh, Gujarat), of these 17 (8.21%) were found EPN positive, and 10 (58.82%)

comprised steinernematids while 7 (41.18%) samples constituted heterorhabditids.

The present study revealed the presence of three EPN species in the area, namely- *Heterorhabditis indica*, *Steinernema thermophilum* and *S. glaseri*. These species are being reported for the first time from north-east region of India, in general, and from the state of Meghalaya, in particular. Studies conducted in India indicate that all these species have also been reported to occur in other regions of India. *H. indica* was originally described by Poinar (1992) from sugarcane fields at Coimbatore (Tamil Nadu). The species had been commonly isolated from subtropical and tropical region of the world such as Sri Lanka (Amarasinghe *et al.*, 1994), Cuba (Joyce *et al.*, 1994), Egypt (Shamseldean and Abd-Elgawad, 1994) and Guadeloupe Island (Constant *et al.*, 1998). In context of India, the species has been reported to occur in Coimbatore and Kanyakumari districts (Ambika, 1995), Kerala (Gulsarbanu *et al.*, 1998) and Bangalore (Hussaini *et al.*, 2000). Recently, Gulsarbanu *et al.* (2005) reported the presence of *H. indica* from Kerela, where its prevalence in the coastal, plain and hilly areas was found to be 7.72, 14.0 and 4.67%, respectively. It was reported that *H. indica* could survive better in sandy soil than soil with loam and clay (Kung *et al.*, 1990). The only other *Heterorhabditis* species that has been reported from India include- *H. bacteriophora*, which was reported from a forest soil in Burliar, Tamil Nadu (Sivakumar *et al.*, 1989). Ganguly and Singh (2000) originally described *S. thermophilum* from the fields of Indian Agricultural Research

Institute (IARI), New Delhi. The species was named 'thermophilum' because of its adaptability to high temperature condition (30–35°C) for its reproduction and multiplication. In this study, *S. thermophilum* was isolated from forest soils having less vegetation bordering Assam, where the temperature ranges from 27–35°C. It may be mentioned here that following Ganguly and Singh's report there has been hitherto no report of the occurrence of *S. thermophilum* elsewhere in India or abroad. The other *Steinernema* sp. encountered in the present study include, *S. glaseri*. This species was originally described from the dead larvae of the Japanese beetle (*Popillia japonica*) from Tavistock Golf Course near Haddonfield, New Jersey (Glaser and Fox, 1930). In this study, the species was recovered from Korhadem forest having sandy loam soil. In India, Gulsarbanu *et al.* (2005) reported the presence of this species from Kerela soils. The present study extends the range of occurrence of this species in India. A perusal of literature reveals an uneven distribution of *S. glaseri* in various regions of the world. For example, Poinar (1979) reported it from United States of America and later from Brazil (Poinar, 1990); De Doucet and Gabbara (1994) encountered it in Spain, Li and Wang (1989) reported it from China, and Stock *et al.* (1997) encountered it in Korea. However, in the northern and western parts of Europe, members of the *S. glaseri* group were found to be absent (Rosa *et al.*, 2000). Similarly, among more than 800 *Steinernema* isolates that were identified to the species level in Germany, none belonged to the *S. glaseri* group (Sturhan, 1999). There is also no record of occurrence of *S. glaseri* in UK, The Netherlands, Belgium and

Scandinavia (Gwynn and Richardson, 1996; Haukeland, 1993; Hominick and Briscoe, 1990; Hominick *et al.*, 1995; Miduturi *et al.*, 1997).

The present study encountered two species of *Steinernema* (*S. thermophilum, S. glaseri*) out of a total of four other steinernematids (*S. abbasi, S. feltiae, S.carpocapsae* and *S. bicornutum*) reported elsewhere in India (Singh, 1977; Ghode *et al.*, 1988; Hussaini *et al.*, 2000; Hussaini *et al.*, 2003). This study therefore adds more information on the diversity of *Steinernema* spp. on Indian soils.

An important indicator determining whether EPNs occur in the environment is the soil type. Soil texture influences nematode survival and mobility. Generally, higher clay content results in lower nematode survival. This is due to decreased pore size and reduced oxygen availability (Molyneaux and Bedding, 1984; Kung *et al.*, 1990). Nematodes are generally more mobile in sandy soil and mobility decreases as the percentage of clay and silt increases (Georgis and Poinar, 1983; Barbercheck and Kaya, 1991). These factors greatly contribute to the distribution of nematodes in particular habitat.

In the present study, all the steinernematids were isolated from sandy loam soil. Similar findings were obtained by Barman *et al.* (1986), Blackshaw (1988), Hominick and Briscoe (1990), Griffin *et al.* (1991), Hara *et al.* (1991), Griffin *et al.* (1994), Mracek *et al.* (1992), Zhang *et al.* (1992), Liu and Berry (1995), Lee *et al.* (1996), Amarasinghe *et al.* (1994), Josephrajkumar and Sivakumar (1997), Midituri *et al.* (1997), Stock *et al.* (1999) who reported a close

association of steinernematids with sandy loam soils. However, Ambika (1995) reported that the nematode occurrence was not related to the soil type. Interestingly, in the present study the *H. indica* was more commonly recovered from red loamy soils near banana plantations in teak forest. In contrast, other studies generally document its frequent occurrence from sandy soils at coastal sites in other subtropical and tropical regions of the world (Burnell and Stock, 2000; Stock *et al.*, 2000). The present study thus adds new information on the soil and habitat preference of this species.

In terms of pH tolerance, in this study the EPNs were isolated from the soil having pH slightly acidic (pH 5.16) to slightly alkaline (pH 7.39). Our findings gain support from Miduturi *et al.* (1996) who reported that EPNs were isolated only in soils having pH between 4.0-8.1. Fischer and Fuhrer (1990) documented that pH < 4.0 can adversely affect nematode host finding. The most favourable pH regarding nematode survival was found to be pH 8 for the species *S. carpocapsae* and *S. glaseri* (Kung *et al.*, 1990). Steiner (1994) reported that *S. kraussei* dominated in soils with relatively low pH values, while *S. intermedia* and *S. feltiae* avoided extremes of pH and *S. affinis* was confined to a pH near neutrality. Miduturi *et al.* (1997) again reported from Belgium that all the EPNs were isolated from soils with a pH range of 3.6-7.8; however, most of the *S. feltiae* populations were isolated in acidic soils. Stock *et al.* (1999) reported that steienernematids were found in sandy loam soils that ranged from acidic (pH 5.0) to neutral. They further mentioned that *H. bacteriophora* and *H. marelatus* were

recovered from loam to sandy loam soils, with a pH ranging from slightly acidic to slightly alkaline (pH 6.3 – pH 7.1). Uribe-Lorio *et al.* (2005) also reported that steinenernematids were isolated from sandy and sandy loam soils with a pH ranging from acidic (5.71) to slightly alkaline (7.24) while *H. indica* was isolated from a loam soil with a pH of 6.84. Various other workers, namely, Mracek and Jenser (1988), Hara *et al.* (1991), Griffin *et al.* (1994) and Hazir *et al.* (2003) also reported the pH of nematode positive soil ranging from 4.6 to 8.0.

In the present study, EPNs were detectable only from forest soils and no nematodes were isolated from other habitats studied. The reason could be attributed to that, the forest trees and shrubs have many defoliators from the orders Lepidoptera, Hymenoptera and Coleoptera, which pupate in the soil and thus serve as the host for EPNs. Similarly, many fly larvae (Bibionidae, Sciaridae and Tipulidae) feed on organic matter and roots in the soil (Mracek and Sturhan, 2000). All these insects may create an ideal environment for EPNs for their persistence and occurrence. In agreement with our findings, Sturhan (1995) reported that *S. silvaticum* prevailed in forest habitats in Germany. Steiner, (1996) also reported the occurrence of *S. intermedium* and *S. kraussei* to be dominant in woodlands. Sturhan (1996) again reported that prevalence of EPNs were highest in woodland (50.5%) and lowest in other habitat ranging from 25.6% to 33.8%. Mracek *et al.* (1999) reported higher number of EPNs positive samples from trees than open habitat. Stock *et al.* (1999) reported that majority

of nematodes were recovered from woodlands (coniferous forest) (33.8%) and oak forests (33.8%) and no nematodes were recovered from soil samples of chaparral or any of the desert habitats. Mracek *et al.* (2005) reported that several species showed a distinct habitat preference; the nematodes occurred frequently (from 57-80%) in the oak, poplar, birch, lime and alder habitats which are especially rich in insects, whereas the occurrence of EPNs was less than 25% in maple, beech and walnut habitats, where insects abundance is low. Mracek *et al.* (1999) also reported that nematodes occurrence was recorded as 67% from habitats that have a high or moderate insect abundance, and only 15% from habitats with less insect abundance. Accordingly, the abundance of insect host seems to be crucial for the EPNs occurrence and distribution. In contrast, Pino *et al.* (1996) recovered EPNs from cropland soils in large number than from woodland and pasture soils, however, the difference between habitat type and EPN population was not significant. EPNs were also isolated from a variety of vegetation types in Malaysia and analysis did not show any association of steinernematids with crop type (Mason *et al.*, 1996). In contrast to the findings of present study, Yoshida *et al.* (1998) reported that *Heterorhabditis* sp. was not restricted by vegetation type; it was isolated from sites ranging from dry open land to wet, well-developed forest. Similarly, Griffin *et al.* (2000) reported that there was no clear association of EPNs with habitat type; both genera were recovered from a variety of vegetation types, both cultivated and uncultivated.

In conclusion, the present study shows that the EPN fauna in the Ri-Bhoi District, Meghalaya, is comprised of three nematode species, i. e., *H. indica, S. thermophilum* and *S. glaseri.* This constitutes the first report of EPNs in the north-east region of India, in general, and in the state of Meghalaya, in particular. In this study, the overall prevalence of EPNs was noted to be 5.37%, and the steinernematids (73.03%) significantly predominated the heterorhabdits (26.97%). Further, the EPNs were found to be present only in forest soils, and no nematodes were detected in dryland, wetland and jhumland (burned and cultivated land). *Steinernema* spp. were mostly isolated from sandy loam soils while *Heterorhabditis* sp., was isolated from red loamy soil. This study also revealed that the EPNs were isolated from the soils having pH slightly acidic (pH 5.16) to slightly alkaline (pH 7.39).

Even though EPN have been proved to be of potential biocontrol agents against a number of insect pests world over, in India it is in developing stage. The nematode species of EPNs exhibit differences in survival, search behaviour and infectivity. Therefore, there is a greater interest in finding populations with traits suitable to local conditions. Although a number of EPN surveys have been conducted in many parts of the world, not much is known about the natural diversity of EPNs in India. There is a need for intensive surveys for isolation of EPN species from different agro climatic zones of India.

The information generated from present study may open the prospects for using these indigenous EPN species in the biological

control programs against insect pests in the area. Indigenous EPNs are suited for the control of local insect pests because they are adapted to the local environmental conditions and are natural regulators of insect populations.

CHAPTER - 2

Seasonal Prevalence of Entomopathogenic Nematodes

Introduction

Entomopathogenic nematodes (EPNs, Steinernematidae and Heterorhabditae) are lethal parasites of various soil-dwelling insects and are being used for the biological control of many insect pests (Hominick and Reid, 1990). They have been used to control insect pests in high value crops and they could be used in large scale in sustainable agricultural systems. Though much research has been done and much progress has been made pertaining to their biology, especially in the last decade, however limited information exists on the persistence of infective juveniles (IJs) of EPNs and their prevalence in natural habitats (Kaya, 1990). Factors that effect EPN population dynamics are still poorly understood. Soil temperature and moisture are known to strongly affect EPN survival and infectivity in the laboratory or controlled field experiments (Kung *et al.*, 1991; Mason and Hominick, 1995), and are supposed to be of great importance for EPN dynamics in the field as well (Kaya, 1990).

The natural habitat for EPNs, the soil, is a difficult environment for persistence of any organism considering its complexity of physical,

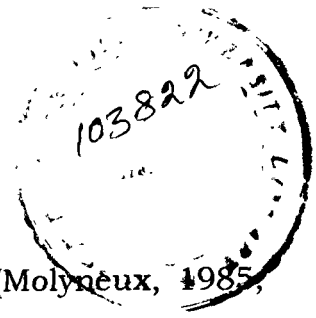
chemical and biological components. Nevertheless, EPNs have been isolated from soils throughout the world in ecosystems ranging from sub-arctic to arid and temperate to tropical climates (Mracek and Webster, 1993; Hominick *et al.*, 1996; Sturhan, 1997).

It has been emphasized that recycling of EPNs is desirable after their application in soil because it can provide additional and prolonged control of insect pests. In natural populations of EPNs, recycling occurs in their insect hosts (Hazir *et al.*, 2003b), but only a few studies have examined the dynamics of nematode population and the factors affecting them. Nematode population is patchy in natural site (Stuart and Gaugler, 1994), biotic and abiotic factors such as seasonal fluctuations, foraging strategy of infective juveniles, host population and alternate hosts (Mracek *et al.* 1999; Mracek and Becvar, 2000) play a key role in nematode recycling.

The extremes of the weather parameters like rainfall, relative humidity and soil temperature are the major limiting factors affecting their persistence (Kaya, 1990). Their persistence has been reported to be good in mild temperatures with adequate soil moisture, wherein gradual desiccation prevails (Prasad *et al.*, 2001b). The IJs ability to disperse and persist until it can locate a host is crucial for the success of entomopathogenic nematode applications for insect control in soil and the survival of naturally occurring EPN populations. Motility and persistence may be influenced by numerous interacting intrinsic factors

(e.g., behavioral, physiological and genetic characteristics) and extrinsic factor of abiotic and biotic nature.

Efficacy of applied EPNs relates closely to the used species and environmental and technical conditions, that no two situations are identical or comparable. Factors of major importance are age and lipid reserves of IJs. These characteristics directly influence the ability of nematodes to survive a period of time without a host and their ability to find and infect a host (Womersley, 1993). Many studies mainly in the laboratories have tested the persistence of EPNs under various conditions in sterile soil. The data generally indicated a survival of weeks rather than months and a gradual decline in the numbers of living nematodes recovered. In some cases, however their infectivity potential does not follow the same pattern because at least some nematode individuals are capable of entering a quiescent state and later become active again when conditions permit host-finding activity (Fan and Hominick, 1991; Womersley, 1993). Behavioural adaptations and phases of anhydrobiosis or quiescence (Womersley, 1993) thus influence the pattern of persistence. However, nematodes in this condition are not pathogenic and only if the environmental conditions are favorable, they retain active and are able to penetrate into insects. The quiescent stage can be induced by extreme temperatures, oxygen deficiency and high salt content (Glazer, 2002).



After application nematodes rapidly disappear (Molyneux, 1985; Kung *et al.*, 1991). Several factors such as soil type (Kung *et al.*, 1990a), humidity levels in soil (Kung *et al.*, 1991), temperatures (Griffin, 1993; Grewal *et al.*, 1994) and soil pH (Kung *et al.*, 1990b) may affect their persistence and infectivity in soil. The temperature tolerance of the IJs depends on their geographical origin. Extreme temperatures are generally unfavourable for the persistence (Kaya, 1990). Nematodes with origin from warmer areas survive better at higher temperatures, while nematodes with origin from cooler regions survive better at cooler temperatures (Kaya, 1990; Curran, 1993; Griffin, 1993).

The long-term persistence of EPN population depends on their ability to find and infect host and produce offspring. In addition to abiotic factors, some biotic factors in the soil also have the influence on their persistence abilities. Biotic factors are natural enemies and intraspesific or interspecific competition and the presence of host insects. Kaya (1990) identified the major natural enemies as collembola, mites, nematophagous fungi, predatory nematodes and microsprodia. Ishibashi and Kondo (1987) pointed out that nematodes have higher survival rates in sterilised soil than in unsterilised soil. Further very scanty information is available in the literature about natural hosts and host specificity of EPNs in nature. Our understanding of EPN population dynamics over the seasons and their possible synchronization with host

life-cycles is also very limited (Smits, 1996). Nowadays, nearly all investigations about EPNs persistence have shown a strong relationship with the soil environments.

The study of seasonal population dynamics of entomopathogenic nematodes, therefore, is fundamental to understand their persistence, distribution and effect on insect populations and for the development of predictive models for control programmes (Hominick and Reid, 1990).

Materials and Methods

Study area

The present study was carried out in the Ri-Bhoi District of Meghalaya. The details about the study area are given previously in Fig. 1.2 of Chapter 1. In brief, the soil in the Ri-Bhoi District may be broadly classified into hill and plain soils. Patches of red loamy soil and lime silt constitutes the major portion. The district experiences different types of climate ranging from tropical climate in the areas bordering Assam to the temperate climate adjoining the East Khasi Hills District. The areas bordering Assam experience hot - humid weather during summer seasons with an average temperature of 30^{0}C, while in other areas like Lum Raitong and Lum Sohpetbneng Plateaus, the climate is severely cold during the winter months.

Site selection and collection/baiting of soil samples

For the study of seasonal population dynamics of EPNs, two nematode positive sites, one for *Heterorhabditis* sp. and one for *Steinernema* sp. were selected in the Ri-Bhoi District, Meghalaya. The soil samples were collected in the morning at monthly intervals for a period of one year from a depth of 10 – 15 cm. Data on soil temperature, soil moisture, ambient relative humidity, and rainfall were collected for the study period.

The seasonal prevalence was studied using indirect method (Prasad *et al.*, 2001b). The presence of EPNs was evaluated by *Galleria* traps/baiting technique (Bedding and Akhurst, 1975; Mracek, 1980). The soil samples were thoroughly mixed and placed separately in 250 ml containers. Ten numbers of last instar larvae of the wax moth, *G. mellonella* were added to each container. The containers were covered and inverted and kept in incubators at 25^{0}C and observed for the insect mortality for a period of 10 days. Three replicates were maintained for each soil sample. First observation on mortality of insect larvae was made on third day after the set up of experiment, and subsequent observations for insect mortality were made daily and continued for a period of ten days. Cadavers were collected and grouped into two; one group was transferred to White traps to collect the emerging Ijs (Woodring and Kaya, 1988), while the cadavers in the other group were dissected and subjected to pepsin digestion in order to collect the adults

(Mauleon *et al.*, 1993). Nematodes were identified up to genus level on the basis of cadaver characteristics (colour of dead insect larvae killed by nematode; brick red in *Heterorhabditids* and pale yellowish in *Steinernema* spp.; Fig. 2.1), and on the basis of male and infective juveniles morphology (Poinar, 1990). The percent larval mortality due to parasitization gave indirect measurement of EPN population. The data on percent mortality of *G. mellonella* were pooled and subjected to simple correlation analysis against weather parameters (Gomez and Gomez, 1976).

Results

The seasonal prevalence of *Heterorhabditis* sp. with respect to soil temperature, soil moisture, rainfall and relative humidity is shown in Fig. 2.2 - 2.3, and of *Steinernema* sp. in Fig. 2.4 – 2.5.

It may be noted that the *Heterorhabditis* sp. was detected in the soil samples throughout the study period, causing moderate to high mortality of the host insect. The prevalence was recorded to be considerably high during the period from March to November. However, comparatively less mortality of insects was observed during December, January and February months, where the soil temperature, soil moisture and rainfall levels were low as compared to the rest of months.

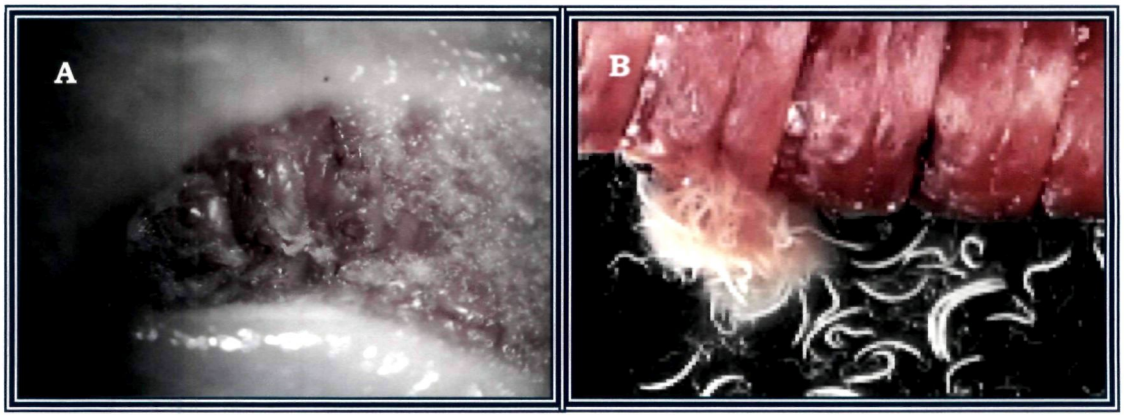
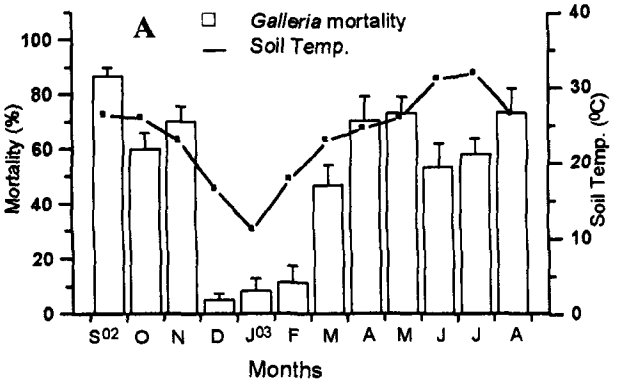
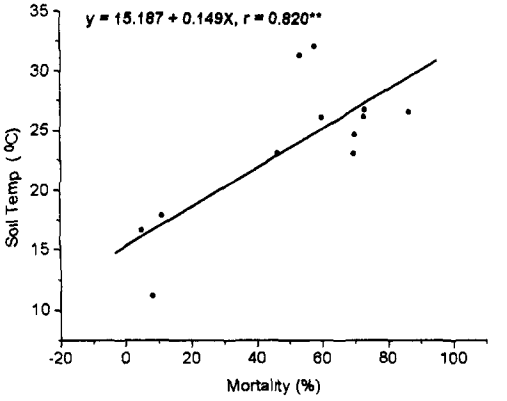
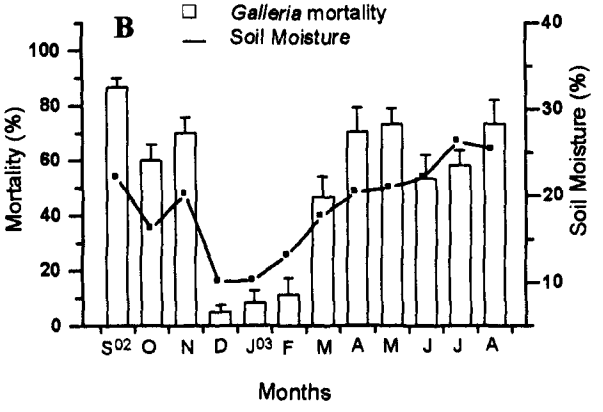


Fig 2.1: A-B; A. Larva killed by *Steinernema* sp. (Pale yellowish)

B. Larva killed by *Heterorhabditis* sp. (Brick red)







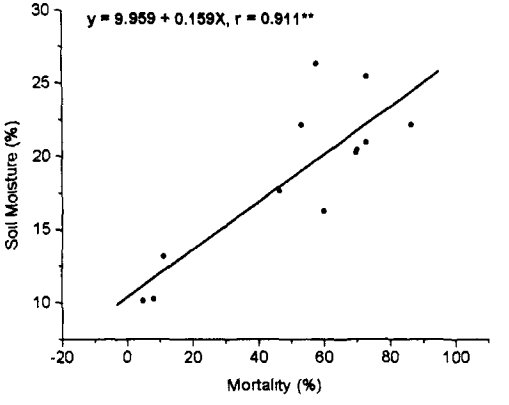


FIGURE 2.2: Seasonal Prevalence of *Heterorhabditis* sp. with respect to:
A. Soil temperature B. Soil Moisture.
*** Significant at 0.01% level*

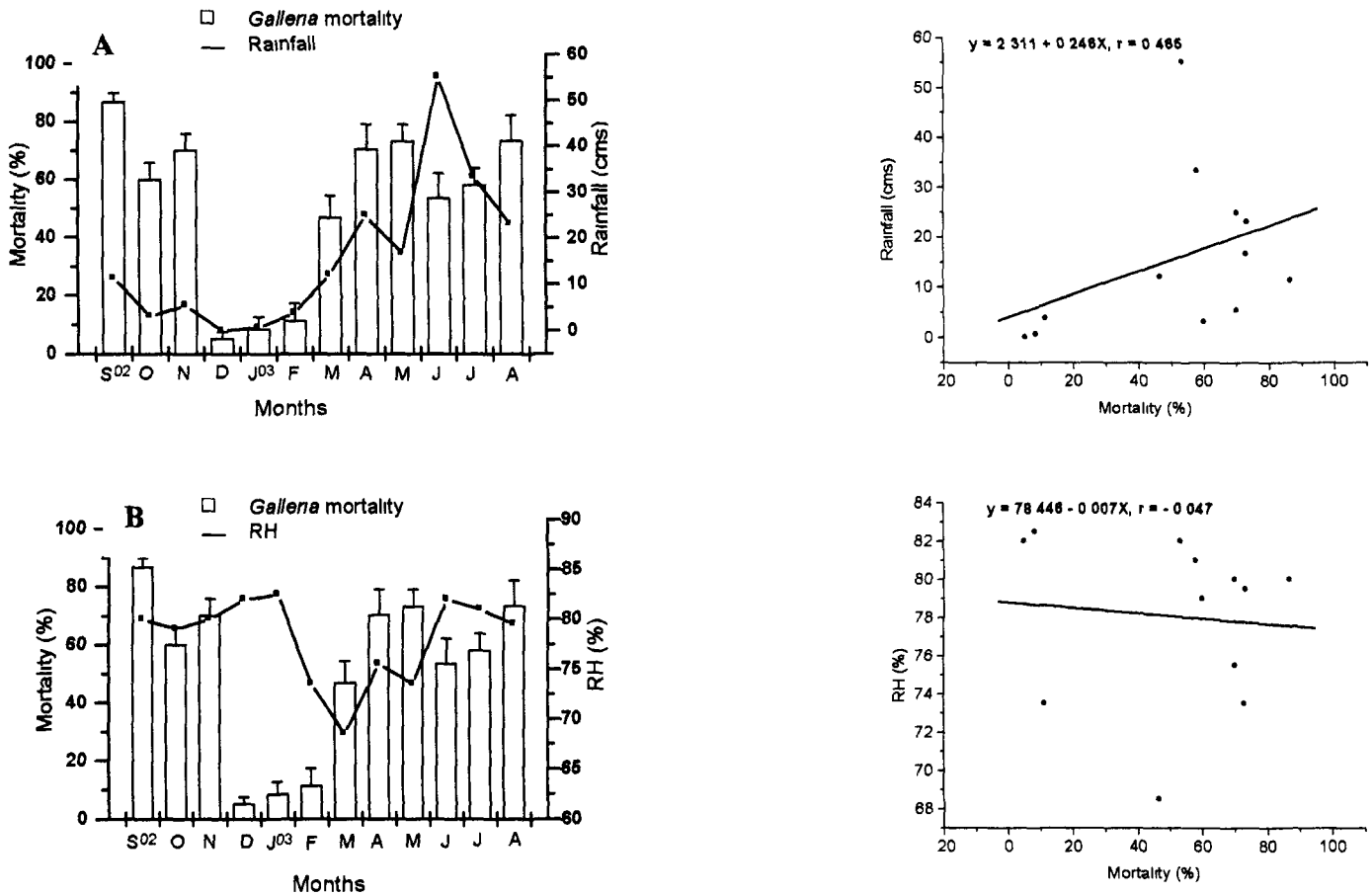


FIGURE 2.3: Seasonal Prevalence of *Heterorhabditis* sp. with respect to: A. Rainfall B. Relative humidity.

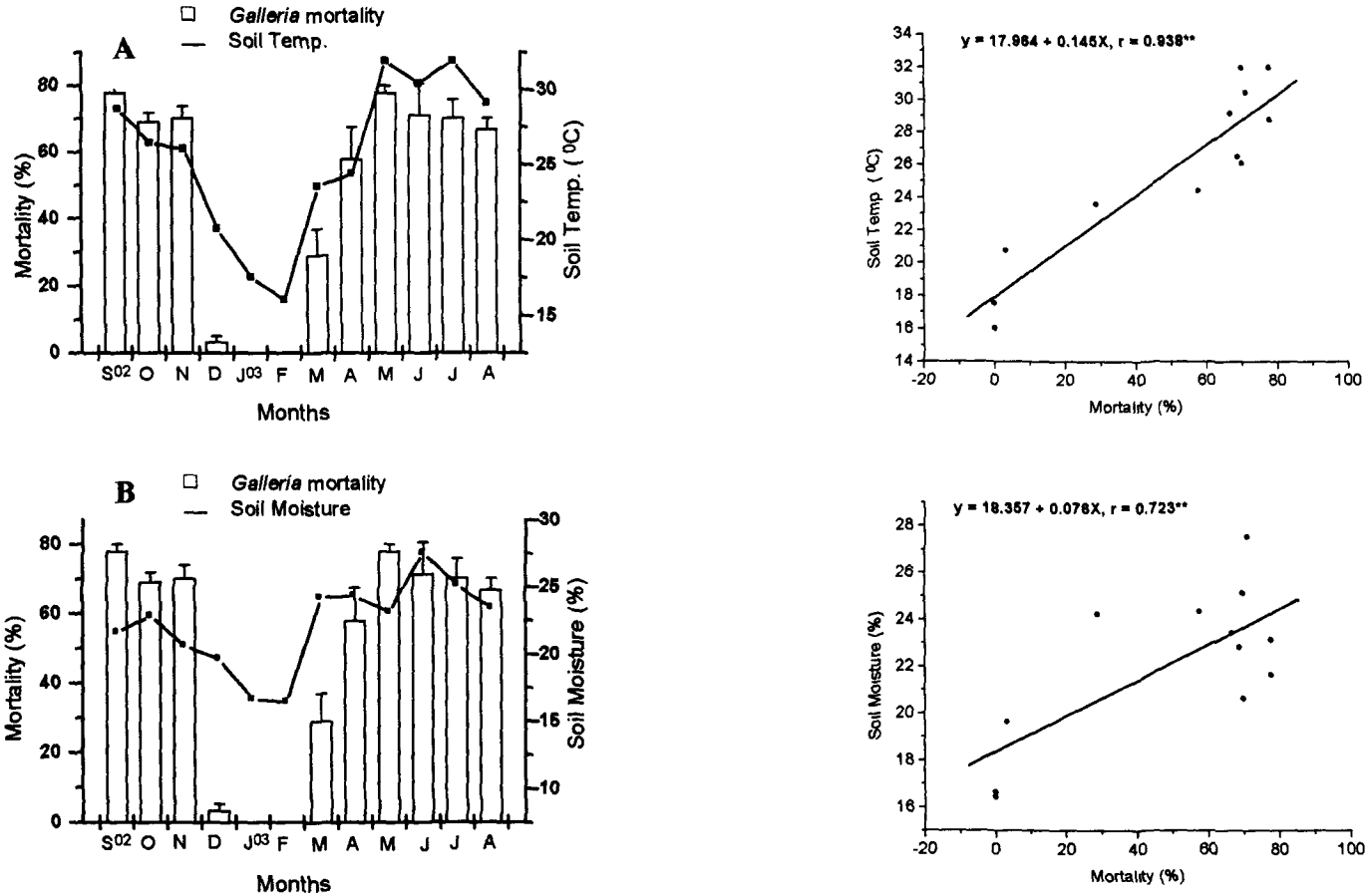


FIGURE 2.4: Seasonal Prevalence of *Steinernema* sp. with respect to:
A. Soil temperature B. Soil Moisture.
** *Significant at 0.01% level*

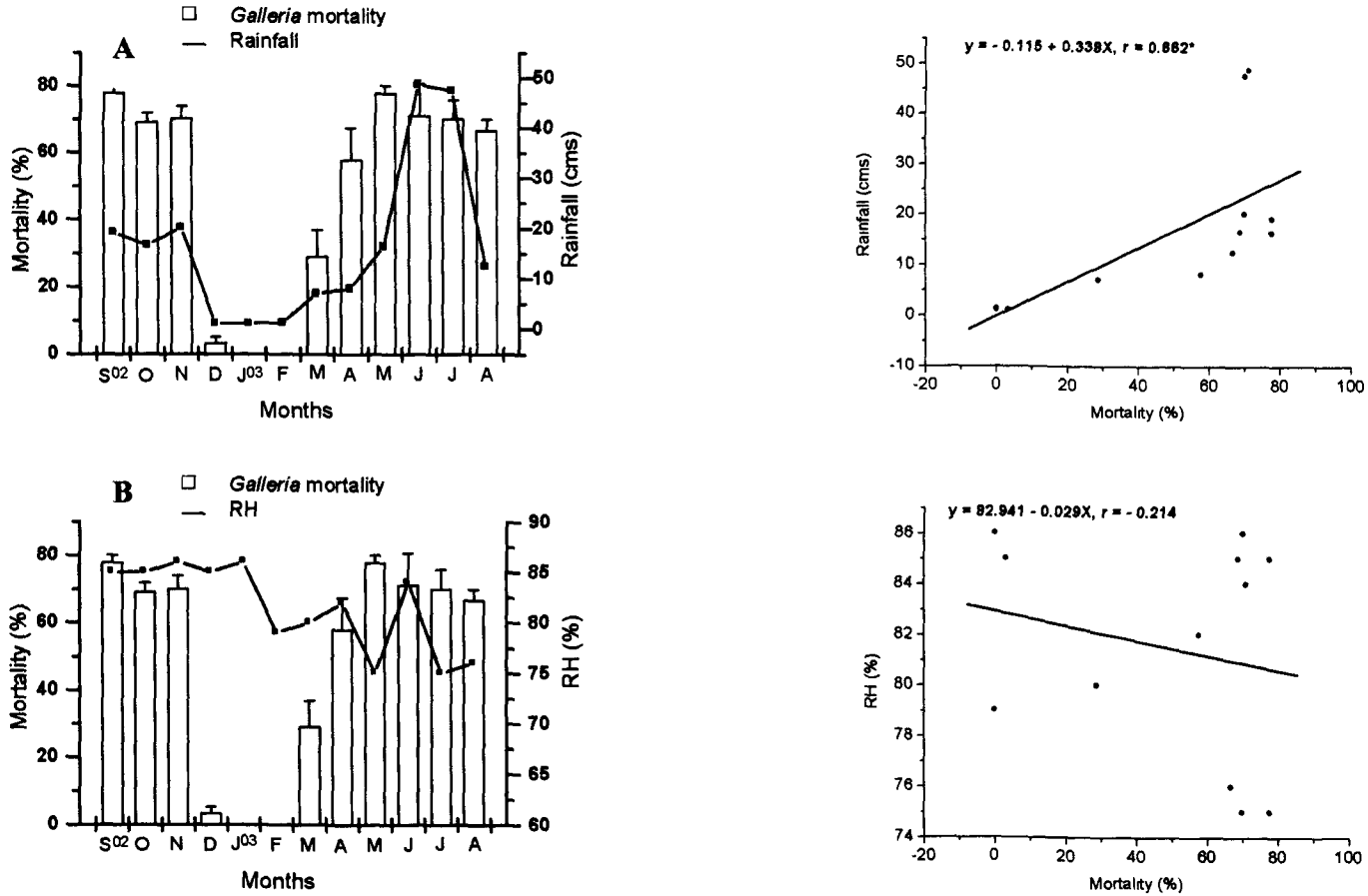


FIGURE 2.5: Seasonal Prevalence of *Steinernema* sp. with respect to:
A. Rainfall B. Relative humidity.
** Significant at 0.05% level*

The prevalence of *Heterorhabditis* sp. was significantly positively correlated with soil temperature (*r* = 0.820**), soil moisture (r = 0.911**) and rainfall (r = 0.465); however, the prevalence of *Heterorhabditis* showed very little or no correlation with relative humidity (r = - 0.047).

The prevalence of *Steinernema* sp. started increasing form March onwards and reached a peak in May; thereafter it maintained almost a uniform trend till November. The prevalence declined abruptly to a very low level in the month of December, and no soil sample was found to be positive for *Steinernema* sp. during the January and February months. The highest mortality of the host insects were observed in May and September months.

The prevalence of *Steinernema* sp. was also found to be significantly positively correlated with soil temperature (r = 0.938**), soil moisture (r = 0.723**) and rainfall (r = 0.662*); however, the same showed a negative correlation with relative humidity (r = - 0.214).

Discussion

The weather parameters such as rainfall, relative humidity, soil temperature and soil moisture have influence on the persistence of entomopathogenic nematodes (Prasad *et al.*, 2001). Soil, the natural habitat for EPNs varies greatly in chemical composition and physical structure. It is a dynamic system in a continual state of flux combined with its physical, biological and chemical complexity; this dynamic state makes the soil a difficult medium in which to conduct quantitative research.

The aim of this study was to study the seasonal prevalence of *Heterorhabditis* and *Steinernema* species with respect to weather parameters, namely soil temperature, soil moisture, rainfall and relative humidity. It is hoped that knowledge of relationships between EPNs and different abiotic factors like soil temperature, rainfall, soil moisture, relative humidity etc. may provide valuable information which may be required to design efficient biological control programme against insect pests in the area.

The present study revealed that both the species show a strong correlation in their prevalence with weather parameters, such as soil

temperature, soil moisture and rainfall. However, no positive correlation was noticeable in their prevalence with respect to relative humidity. In case of *Heterorhabditis* species the prevalence was observed to be moderately high during March to November which coincided with a moderately high soil temperature and soil moisture. Almost a similar trend was observed for *Steinernema* sp., except that its prevalence was noted to be negligibly low in the month of December and the species was not detectable at all during the months of January and February. A possible explanation for the same may be relatively low rainfall during this period which resulted in to low soil moisture. In this study the steinernematids were isolated from sandy loam soil. The water retention capacity of sandy loam soil is comparatively low than soil rich in clay contents. It is a well recognized fact that the dry conditions adversely affect nematode motility and host seeking behaviour (Menti *et al.*, 1990). The potential of EPN to survive desiccation is generally reported to be poor (Glazer, 2002). Surrey and Wharton (1995) tested the desiccation survival of *H. zealandica* and noted that its survival was poor once water had been lost from the substrate. Menti *et al.* (1997) showed that although *H. megidis* survival was superior to that of *S. feltiae*, desiccation tolerance for both species was poor. Thus desiccation must be considered as one of the limiting factor in the occurrence of EPNs in soil.

Environmental conditions can have tremendous influence upon survival rates of EPNs. Soil temperature is one of the important factors determining the abundance of nematodes in soil. Temperatures above 40°C and below 0°C are lethal for most EPN species (Grewal *et al.*, 1993). Griffin (1993) and Grewal *et al.* (1994) have reviewed the temperature ranges for survival and reproduction of EPNs and reported that higher temperatures increase the rate of metabolism of nematode and shorten its life span. In the present study, the prevalence of *Heterorhabditis* sp. and *Steinernema* sp. was recorded to be significantly positively correlated to the soil temperature. Such a phenomenon of positive relationship was also reported by Beavers *et al.* (1983). In general, in most of the studies made in the tropical and sub-tropical regions of the world it has been invariably noted that mild temperature favors survival of entomopathogenic nematodes better (Kaya, 1990). Soil temperature is determined to large extent by factors which control transfer of heat in and out of the soil. Wet soil has a greater conductance and smaller rise in temperature than dry soil with the same input of heat at the surface. The solar heat penetrates deeper in wet soil, but produces a smaller rise in temperature than in dry soil. The deeper layers are more buffered than the surface, which tends to heat and cool rapidly along with the atmosphere and under the influence of direct sunlight (Kaya, 1990). It may be mentioned here that comparatively less prevalence of EPNs observed during the winter

(December to February) may be due to the characteristics of EPN species which were warm adapted.

The present study could not reveal any significant correlation between prevalence of EPNs with relative humidity. The survival of EPNs at comparatively low relative humidity may be due to the retention of second stage cuticle by the infective juveniles, thereby slowing down their rate of drying. Apart from soil moisture, texture and porosity also influence the relative humidity of the soil (Kaya, 1990). The sandy soils have large pore space but less total pore space, than loam or clay soils (Prasad *et al.*, 2001b). Puza and Mracek (2004) studied seasonal dynamics of indigenous EPNs in meadow and oak forest habitats and recorded high nematode densities at the beginning of the season followed by a rapid decrease and stabilisation during the winter. They also pointed out that nematode abundance was significantly correlated with the abundance of suitable insect hosts. Thus a low prevalence of EPNs during winter as recorded in the winter months may also be due to nematodes overwintering in insect bodies. Insect populations build up during summer and as a result, nematode population increase in late summer and in autumn.

Studies made in Florida citrus groves, revealed that the *Steinernematids* and *Heterorhabditids* were recovered from the soil most often from May to November, wherein a moderately high soil

temperature prevailed. Almost similar pattern were observed in the present study, where *Heterorhabditis* sp. and *Steinernema* sp. were recovered regularly during March to November coinciding with warm temperature and well distributed rainfalls which maintain required conducive moisture in the soil. In the present study a positive correlation was observed between rainfall and the prevalence of *Heterorhabditis* sp. (r = 0.0465) and *Steinernema* sp. (r = 0.662*). These findings are in agreement with similar kind of trends reported by Prasad *et al.* (2001b), who reported that the prevalence of *Heterorhabditis* sp. was significantly positively correlated with rainfall (*r* = 0.92**). The prevalence was influenced by rainfall to the extent of 92%, the rest being contributed by other factors. Similarly, Josephrajkumar and Sivakumar (1997), while surveying EPNs from Tamil Nadu (India), reported that the presence of *Steinernema* sp. was related to rainfall and is confined to the south-west monsoon and north-east monsoon season. They further reported that when rainfall and frequency of occurrence of *Steinernema* sp. is considered, the hilly tract with higher rainfall and more number of rainy days register a higher frequency of nematode occurrence.

Strong (2002) in a study on food webs in a bush lupine ecosystem with the lepidopteran species *Hepialus californicus* and *H. marelatus* as a naturally occurring antagonist show a strong relation between the availability of host insects and the nematode population. Mracek and

Becvar (2000) also advocated that one the major factor on long term persistence of EPNs is the presence of host insects and thus indirectly the host plant, providing a basis for the insect population. The presence of a suitable host within traveling distance is a critical parameter for the survival of a nematode population. The relation between insects and nematode population thus is well established. Soil temperature, humidity, acidity and soil type mainly have their impacts when extremes are reached which can dramatically reduce an EPN population (Kung *et al.*, 1991). However, the weather conditions have a more immediate impact on host finding capability of infective juveniles. Grewal *et al.* (1994) and Kung *et al.* (1991) reported that EPNs infectivity is affected by many environmental conditions, including soil temperature and moisture. For example, Grewal *et al.* (1994) found that nematodes which were isolated during December, when soil temperatures were cooler than 10^0C, were not active in the field.

It may therefore be concluded from this study that extremes of the weather parameters like soil temperature, moisture and rainfall are the major limiting factors affecting the persistence of entomopathogenic nematodes in soil. The study shows that persistence of EPNs is good in mild temperatures with adequate soil moisture, wherein gradual desiccation prevails.

CHAPTER – 3

Ecological Characterization and Bio-efficacy of EPNs against Insect Pests

Introduction

Isolates of EPNs from specific regions exhibit considerable variations in terms of their host range, reproduction, infectivity and conditions for survival (Bedding, 1990). The biocontrol potentials of EPNs are therefore influenced by different abiotic and biotic factors, beside others (Kaya, 1990; Koppenhofer *et al.*, 1995). Although detailed knowledge of the ecology of biological control agents is essential for their successful use, our knowledge about the majority of EPN species extends little beyond their taxonomic description (Navon and Ascher, 2000). Most species have been isolated from soil samples using larvae of greater wax moth *G. mellonella,* and their natural hosts are rarely known (Koppenhoper *et al.*, 2000).

Experiments studying the efficacy of EPNs to control insect pests have often yielded variable results because the biology of pests and the nematodes, including IJs behavior and infection strategy was not sufficiently taken into consideration (Gaugler *et al.*, 1994).

It has been reported that the temperature plays an important role in affecting the infectivity, time of death, development, reproduction and storage of EPNs (Mracek *et al.*, 1997; Koppenhofer and Kaya, 1999;

Ganguly and Singh, 2001). Similarly, one of the important steps in the utilization of EPNs for biocontrol programme is the storage of the infective juveniles in a given population density at optimum temperature, so that maximum IJs can survive for long periods before they are utilized for field application.

Soil moisture is also considered as one of the important factors (Kaya, 1990). It has been reported that the low soil moisture can adversely affect nematode activity and survival, but a considerable number of terrestrial nematode species could survive some degree of dehydration if the drying process is gradual (Womersely, 1987).

It is for these reasons that for their use in biological control, locally adapted species or isolates from native habitats need a characterization in terms of their optimum biological requirements (Stock *et al.,* 1999).

With the increasing concern over pesticide resistance in insects and residues in environment, Biointensive Integrated Pest Management (BIPM) has emerged out as an important strategy for control of insect pests. In the present scenario none of the strategies can be used as a sole component for successful management. Use of chemical pesticides and biocontrol agents in IPM has received much interest in recent years (Ignacimuthu and Jayaraj, 2003). The entomopathogenic nematodes, a highly competitive biocontrol agent have been used successfully to suppress insect pests in several crops (Grewal *et al.*, 2005). For any

biocontrol agent to fit into IPM strategy, it should be compatible with other methods of control.

One of the major constraints in agriculture production in India is losses sustained due to attack by pests and diseases. The agricultural pests include mites, disease causing pathogens, weeds and other organism causing damage to crops, but in many instances it has been used to denote insects alone. Insect pests are important, as more than one third of damage to the crop caused by various pests is attributed to the insects and mites. One or the other insect pests are always associated with every crop grown, but not all these pests are of economic importance. When the pest abundance crosses the action threshold or the economic injury level, their control is one of the main agricultural requirements for increase in crop productivity.

Haplosonyx chalybaeus (Hope) (Coleoptera: Chrysomelidae) is a serious pest of colocasia (a vegetable crop locally called as 'yam' in Meghalaya). The life cycle of *H. chalybaeus* is shown in Plate 3.1. The adult beetles feed on the leaves by making circles of different sizes. The female lay eggs in the shoot, leaf sheath above the ground. After hatching, the grub bores in the shoot and finally feeds on the developing rhizome. Upto 70-80 larvae per plant may feed on rhizomes (Shylesha, 2003).

The cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) is a common pest of cruciferous including broccoli, brussel, sprouts,

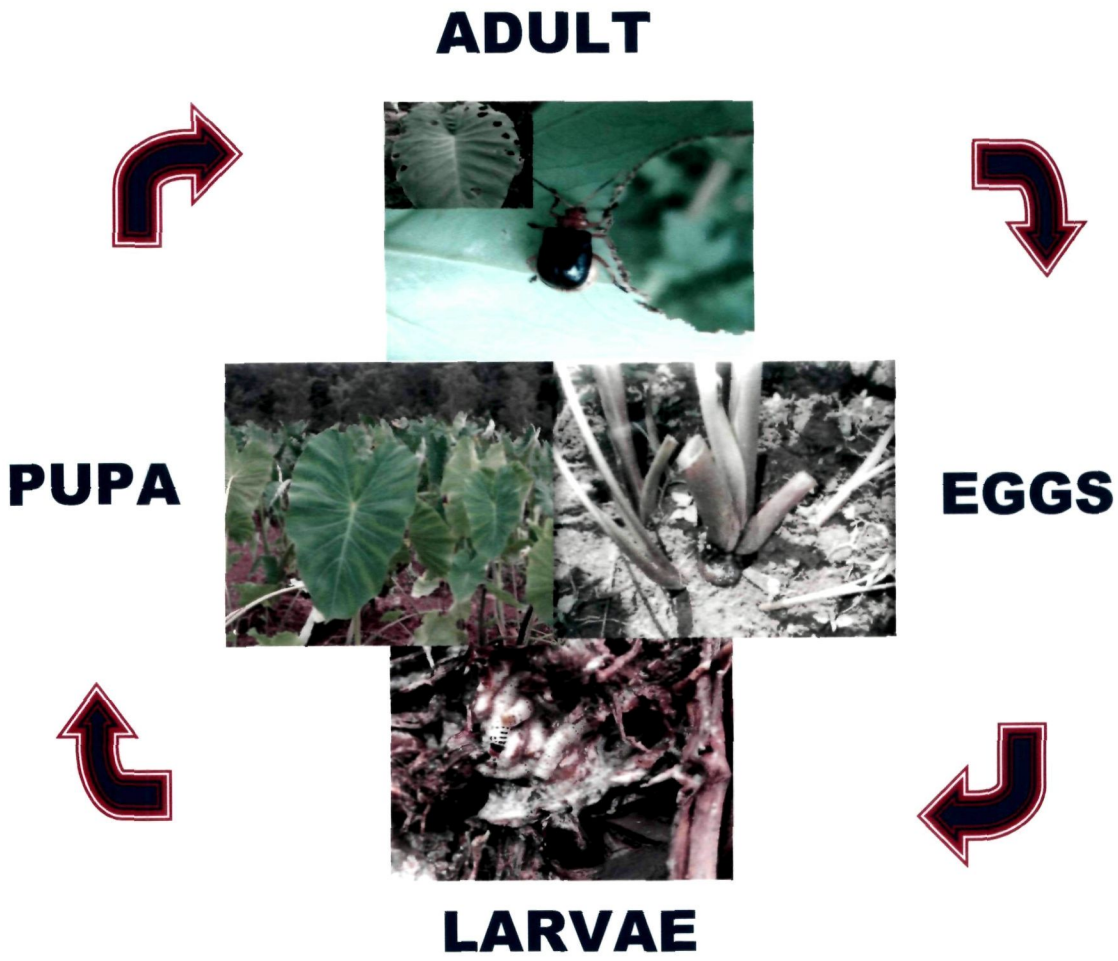


PLATE 3.1: Life cycle of *Haplosonyx chalybaeus*

cabbage cauliflower and other crops in Meghalaya. The life cycle of *P. brassicae* is shown in Plate 3.2. Young larvae graze away the lower epidermis of the leaves whereas the older larvae cause extensive defoliation and often reduce plants to a skeleton of stems and major veins; it may also kill the plant. Besides, the plants are also contaminated with large quantity of its faeces (Hill, 1987; Alford, 1990). The larvae pass through five instars and feed gregariously; and are fully grown in about 24 days. They leave the plant to pupate on a solid substrate nearby such as wall, fence, tree trunks etc. Pupation takes 10 -15 days and second generation emerges. Eggs are laid in batch of 20 – 100 mostly on the underside of the leaves. They hatch about 1 – 2 weeks later depending on the temperature (Ramadhane and Ihsan, 1999).

The brinjal fruit and shoot borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae), is the major insect pest of eggplant, *Solanum melongena,* throughout Asia, including Meghalaya, causing 25 – 40% damage to brinjal fruit yield (Hussaini *et al.*, 2002). Plate 3.3 depicts the life cycle of *L. orbonalis*. The female laid eggs on the underside of the brinjal leaves. After hatching, larvae bore into shoots during the vegetative growth stage and later in flowers and fruits, rendering fruit unfit for human consumption. At the time of pupation the larvae moves and pupate to the fallen leaves or to the shoot of the host plants.

The mustard saw fly, *Athalia lugens proxima* Klug (Hymenoptera: Tenthridinidae) is a serious pest of radish and mustard in north-eastern

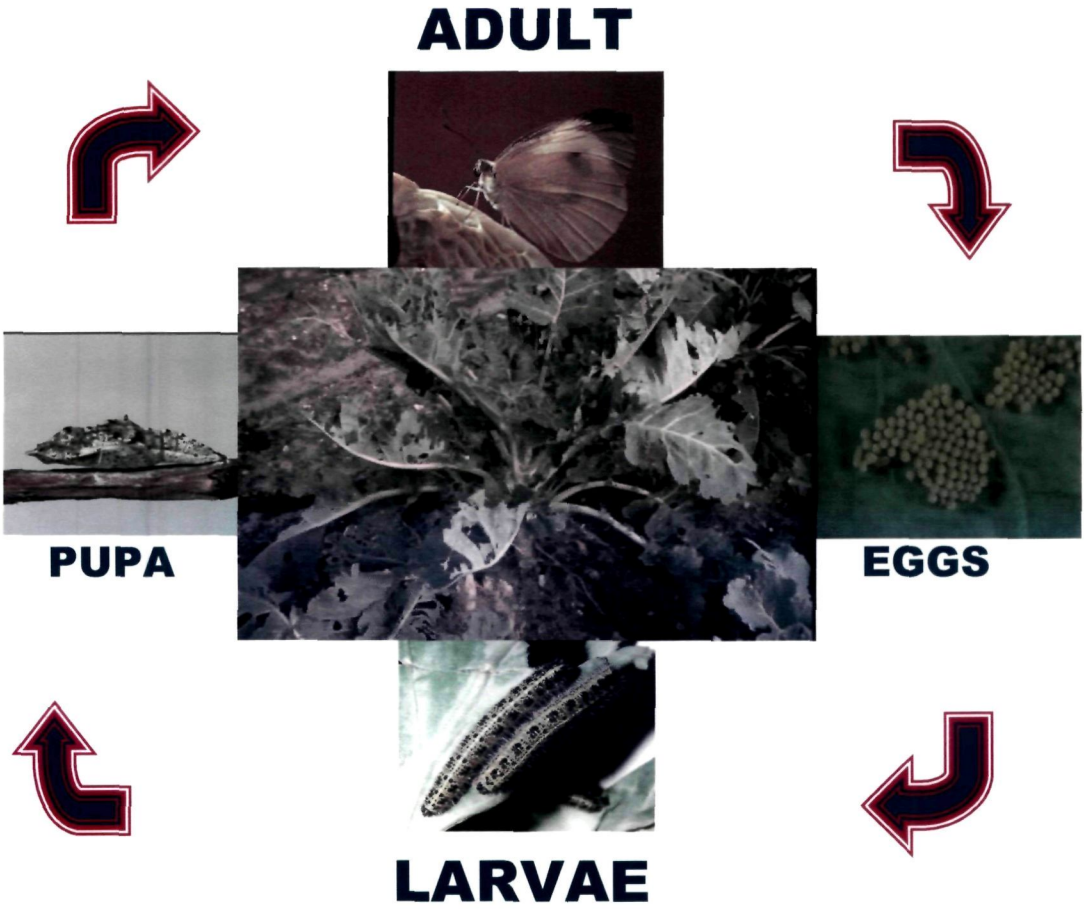


PLATE 3.2: Life cycle of *Pieris brassicae*

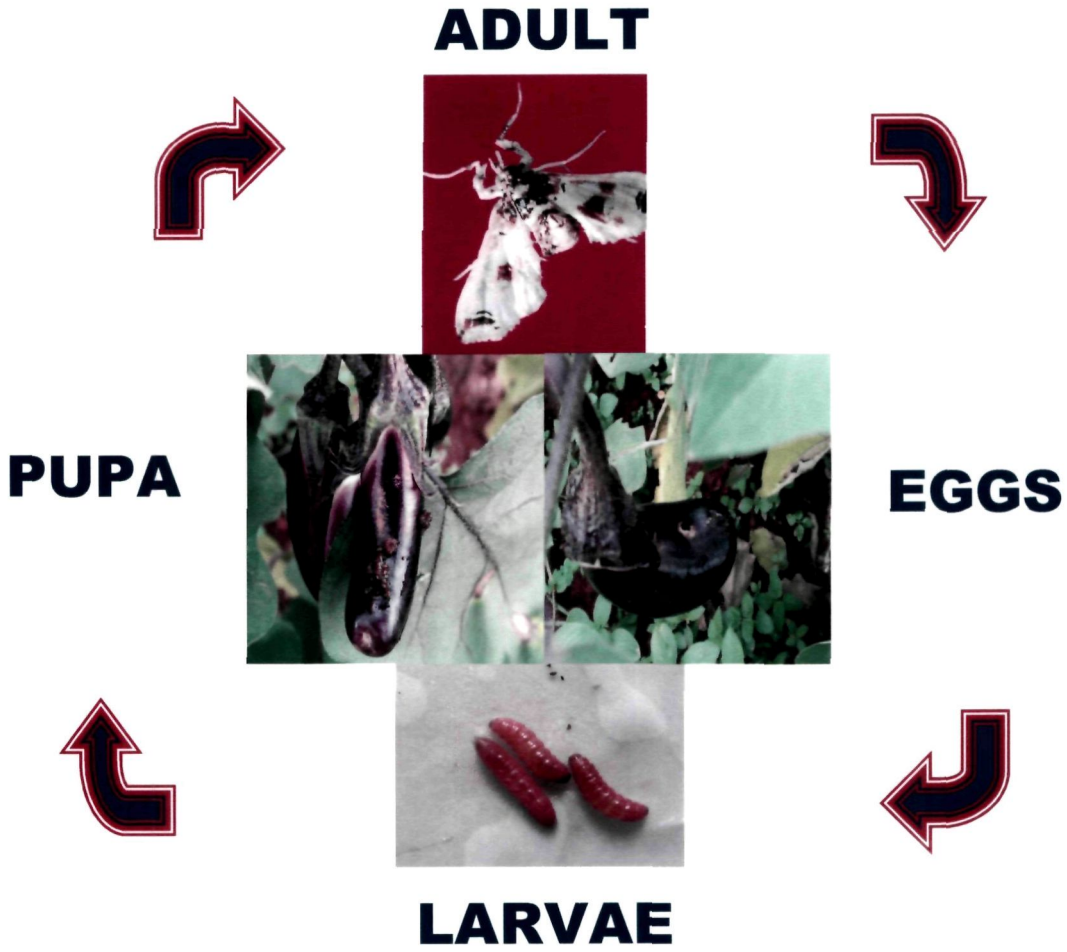


PLATE 3.3 : Life cycle of *Leucinodes orbonalis*

region of India (Shylesha, 2003). The life cycle of *A. lugens proxima* is illustrated in Plate 3.4. The adult oviposits inside the basal stem of the leaves. When hatched, larvae feed on the leaves and flowers. After 17 - 20 days, larvae move towards the soil or fallen leaves and pupate. After 10 – 12 days the adults emerge and continue their life cycle.

Rapidly increasing knowledge regarding biology, host range and epidemiology has laid groundwork for the eventual use of EPNs as effective biological control agents world-wide. In developed countries like USA, Australia and Europe, commercial nematode based products are available and are being utilized for biological control of insects (Grewal *et al.*, 2005). EPNs have emerged as excellent candidates for biological control of insect pests. Attributes making the nematodes ideal biological insecticides include, their broad host range, high virulence, safety for non-target organisms and high efficacy in favorable habitats. Progress achieved in liquid fermentation, formulation stability and application strategy has allowed nematode-based products to become competitive with chemical insecticides in medium and high valued crops on the basis of cost/benefit ratio and ease of application. One of the fundamental steps in development of an EPN for biocontrol is choosing an appropriate strain. Virulence against the target pest is a basic factor of biological control programme. The characterization of traits related to

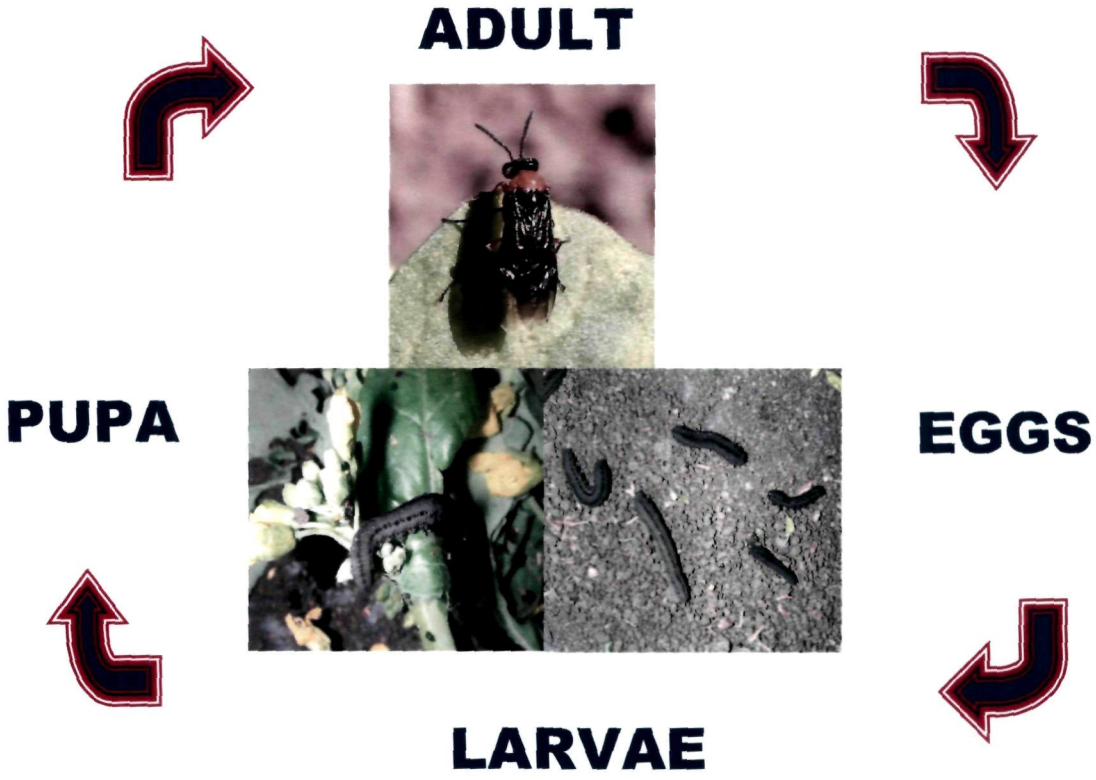


PLATE 3.4: Life cycle of *Athalia lugens proxima*

the control potential of species (strains) of entomopathogenic nematode is therefore the key for a successful biological pest control in an area.

The objective of the present study was to provide basic information necessary for the development of EPNs isolated in this area as biological control agents. The study incorporates ecological characterization of three nematode species *(H. indica, S. thermophilum* and *S. glaseri*) and their bioefficacy against selected insect pests under laboratory conditions.

Materials and Methods

In the present study the EPNs used for ecological characterization and bioefficacy tests include, *H. indica, S. thermophilum* and *S. glaseri* which were isolated from the Ri-Bhoi District of Meghalaya and reared *in vivo* on larvae of the wax moth, *Galleria mellonella,* according to Woodring and Kaya (1988). The ecological characterization of nematodes was made using *G. mellonella* as a model insect host. While the bioefficacy tests were carried out against four economically important insect pests in the area, namely- *Haplosonyx chalybaeus, Pieris brassicae, Leucinodes orbonalis* and *Athalia lugens proxima.*

A. *Ecological characterization of EPNs*

1. Effect of temperature on:

i) *Infectivity of IJs:*

Infectivity of IJs was determined by the number of nematodes established per insect larva at different temperatures, using methods of Woodring and Kaya (1988), with slight modifications.

Petridishes (35 x 10 mm) were lined with double layer of Whatman No. 1 filter paper. 100 IJs of nematodes in 0.5 ml of D.W. were evenly distributed on filter papers. Petridishes were transferred to incubators set to different temperatures *viz,* 10, 15, 20, 25, 30 and 35 ± 2^{0}C , and kept for at least half an hour. One *G. mellonella* larva was placed in each of the petridishes and sealed with parafilm. Eight petridishes were prepared for each temperature. The petridishes were checked for larval mortality. The dead larvae were collected 2 days after inoculation for *Steinernema* spp. and 3 days after inoculation for *H. indica.* Larvae were dissected and subjected to pepsin digestion (Mauleon *et al.*, 1993) to record the establishment of IJs per larva. To each temperature, one petridish, prepared as described above but without IJs was kept as control.

ii) *Reproduction:*

The effect of temperature on reproduction of nematodes was studied according to the method of Brown and Gaugler (1997), with slight modifications. Last instar *G. mellonella* larvae were killed by nematodes @ 100 IJs/larva at 25 ± 2°C by petridish assay.

The infected dead larvae were set singly on white traps and transferred to incubators adjusted to 10, 15, 20, 25, 30 and 35 ± 2°C temperatures. Observations were made after 3 days from inoculation at every 24 hrs interval to record the day when the IJs first emerged from the cadavers. Total productions of IJs were recorded for 20 days period in each case. Eight replicates of insects were used for each temperature.

2. Effect of relative humidity on reproduction:

The effect of relative humidity on reproduction of nematodes was studied according to the method of Brown and Gaugler (1997), with slight modifications. Last instar *G. mellonella* larvae were killed by nematodes @ 100 IJs/larva at 25 ± 2°C for *H. indica* and *S. glaseri,* and at 30 ± 2°C for *S. thermophilum* by petridish assay.

The infected dead larvae were set singly on white traps (White, 1927) (Plate 3.5), and transferred to BOD incubators

PLATE 3.5: White traps showing arrangement of *G. mellonella* larvae to collect EPN IJs.

adjusted to 70, 80, 90 and 100 ± 5% R.H. at 25 ± 2°C for *H. indica* and *S. glaseri* and at 30 ± 2°C for *S. thermophilum.* Observations were made after 3 days from inoculation at every 24 hrs interval to record the day when the IJs first emerged from the cadavers. Total productions of IJs were recorded for 20 days period in each case. Eight replicates of insects were used for each relative humidity.

3. Storage and persistence:

The storage and persistence of IJs was studies as per the method of Karunakar *et al.* (2001), with slight modifications. Freshly emerged (3 to 5 days after first day of emergence) IJs of each nematode species were collected from white traps and subjected to their storage and persistence activity.

The population densities were adjusted to 100, 500 and 1000 IJs/ml in 20 ml of D.W. in 50 ml conical flask. The conical flasks were stored in BOD incubators at 5, 10, 25 and 30 ± 2°C for 120 days. 3 replicates were maintained for each temperature and time. The survival and pathogenicity of IJs were checked at 15, 30, 60, 90 and 120 days after storage in each temperature. Survival of IJs was determined under a dissecting microscope by drawing 1 ml of suspension from each density and temperature. Immobile IJs were touched with a fine probe and those that did not react were recorded as dead. Pathogenicity test was done by petridish assay with 8 numbers of *G. mellonella* larvae @ 100

IJs/larva. Larvae were subjected to pepsin digestion to record the number of IJs established/larva.

4. Foraging behavior:

i) *Attachment of IJs to the insect host:*

The method followed was adopted from Koppenhofer *et al.* (2000). The bottom of petridish (100 x 15 mm) was lined with one moist filter paper that was sprinkled with 1 gm of sand. Approximately 1000 IJs were distributed in the dish in 0.5 ml of D.W. After 30 minutes, one wax moth larva was introduced in to each petridish. The larva was kept crawling continuously by disturbing it with a prod whenever it stopped. After 1, 5 and 10 minutes, the larva was recovered, rinsed with D.W. in a petridish, numbers of IJs in the rinse was counted under dissecting microscope. There were 8 replicates for each species.

ii) *Effect of soil depth:*

The method of Koppenhofer *et al.* (1995), with slight modification was followed in this experiment. A hole was made at the bottom of a plastic container (5 cm diameter and 12 cm height) and covered with a wire mesh. A petridish (5 x 3 mm) was filled with soil (15% w/w) and a depression was made on the surface. 8 numbers of wax moth larvae were put in the petridish and the plastic container was put on top of it. The plastic container was then filled with 15% moisture (w/w) soil to make it

to 2, 5 or 10 cms high. Approximately 2000 IJs in 1 ml of D.W. were applied on the surface of soil and covered it with a lid. The container was transferred to the incubator set at 25 ± 2°C in case of *H. indica* and *S. glaseri*, and at 30 ± 2°C for *S. thermophilum* and later disassembled after 4 days. The wax moth larvae, dead or alive, were rinsed with D.W. Following this, the dead larvae were subjected to pepsin digestion and the lived ones were placed in a cleaned petridish lined with a wet filter paper for another 2 days. Larvae that died afterwards were dissected 1 day after their death to determine the nematodes establishment. There were 3 replicates (containing 8 insects each) per soil depth.

5. Effect of soil moisture on establishment of IJs:

Method of Koppenhofer *et al.* (1995) was followed with slight modifications. The experiments were conducted at 25 ± 2°C (30 ± 2°C for *S. thermophilum*). A sandy loam soil (85% sand, 12% silt and 3% clay, pH 6.54) was used which had been autoclaved at least 2 weeks before use. Freshly emerged IJs were used that were harvested from White traps. The experiments were conducted in 24-well tissue culture plates (3 ml arena). The soil moisture levels tested were 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 25% (w/w). For each moisture level 100 IJs in 25 µl of D.W. were placed on the bottom of each well, which was then filled with pre-wetted soil so as to give desired moisture levels. A tiny depression

was made on top of the soil to accommodate one insect larva in each well. The plate was covered, sealed with a cellophane tape and turned upside down. During the exposure period the plates were flipped upside down every 8 hrs to check the upward movement of insect larva and to achieve uniform distribution of IJs. After 3 days insects were recovered from each well and the numbers of dead insects were recorded. They were rinsed in water, dissected and subjected to pepsin digestion. The number of IJs established/insect host was recorded. To each moisture level, wells prepared as described above but without nematodes, was kept as control for checking insect mortality. There were 8 replicates of *G. mellonella* per soil moisture level for each EPN species.

6. Effect of population density on reproduction:

The method of Selvan *et al.* (1993) was followed. 24 well (3 ml arena) tissue culture plates were lined with filter paper. Freshly emerged isolated nematodes were put at a concentration of 10, 20, 50, 100, 200, 500, 1000, 2000, 3000, 5000 and 8000 IJs in 0.5 ml of D.W. After 30 minutes, one wax moth larva was added at each dosage. Each concentration was replicated 16 times. After 3 days larvae were recovered and rinsed in D.W.

Dead insects were divided into 2 groups (8 nos. each). One group was subjected to pepsin digestion to record the number of

IJs established. The other group was set singly on white traps to count the larvae. Observations were made at every 24 hrs interval to record the day when the IJs first emerged from the cadaver. Total production of IJs was recorded for 20 days period in each case.

7. Effect of chemical pesticides on the survival and pathogenicity:

Chemical pesticides used in the study were Carbaryl (Lower conc.: 1 gm/500; Higher Conc.: 2 gms/500 ml), Nimbecidine (Lower conc.: 1 ml/500 ml; Higher Conc.: 2 ml/500 ml), Endosulfan (Lower conc.: 0.07%; Higher Conc.: 0.15%), Quinolphos (Lower conc.: 0.05%; Higher Conc.: 0.1%), Fenvalerate (Lower conc.: 0.05%; Higher Conc.: 0.1%), Mancozeb (Lower conc.: 1 gm/500 ml; Higher Conc.: 2 gms/500 ml) and Carbofuran (Lower conc.: 1 gm/500 ml; Higher Conc.: 2 gms/500 ml). All pesticides were tested at 2 concentrations (one higher and one lower of the recommended field rates) (Pathak *et al.*, 2001). Stock solutions of the pesticides were prepared in D.W. at double strength of the required concentration. About 1500 IJs of the nematodes in 2.5 ml of D.W. were mixed with 2.5 ml of each chemical stock solution in a petridish and incubated at 25 ± 2^{0}C for 72 hrs. One petridish filled with about 1500 IJs in 5 ml of

D.W. but without any pesticide served as control. There were 8 replicates for each pesticide.

After 72 hrs of incubation, nematode suspensions from each pesticide and concentrations were centrifuged at 600 rpm for 3 minutes separately. The resultant supernatant was decanted and replaced by D.W. This procedure was repeated several times. The numbers of IJs survived were counted. Nematodes that did not move even after prodding were considered dead (Hussaini *et al.*, 2001).

Washed IJs were evaluated by Petridish assay method at a concentration of 100 IJs/larva against *G. mellonella* larva (8 replications). After 3 days the larvae were recovered and rinsed in D.W. and subjected to pepsin digestion to record the numbers of IJs established.

B. *Laboratory evaluation of EPNs against insect pests*

The bioefficay of three EPNs, *H. indica*, *S. thermophilum* and *S. glaseri* was tested against the four insect pests, Colocasia corm borer, *Haplosonyx chalybaeus* Hope (Coleoptera: Chrysomelidae), Cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae), Brinjal fruit and shoot borer, *Leucinodes orbonalis* Guene (Lepidoptera: Pyralidae) and Mustard sawfly, *Athalia lugens proxima* Klug (Hymenoptera: Tenthridinidae).

All the insects' larvae were collected along with their natural diets from Indian Council of Agriculture Research, Umiam experimental farms and kept for at least 5 days in the laboratory to check, whether or not, there are any other infections before using them for experiments. To collect the pupal stage, the larvae were reared in the lab with their natural diet till their life-cycle reached the pupal stage.

i) Mortality Test for Larvae

Eight nos. of petridishes (35 x 10 mm) were lined with double layer of Whatman No.1 filter paper for each nematode concentration. Nematodes of different concentrations (25, 50, 75, 100 and 200 IJs/larva for *H. chalybaeus* and 10, 25, 50, 75, and 100 IJs/larva for others insect pests in 0.5 ml of D.W.) were evenly distributed on the filter paper and kept at least for 30 minutes. One larva was placed in each of the petriplates and sealed with parafilm. Number of insect pest larvae showing mortality were recorded every 24 hrs till 120 hrs. Larvae placed on wetted filter papers without IJs served as control. 3 replicates (containing 8 insects each) for each nematode species and concentration were set.

ii) Mortality Test for Pupae

Eight nos. of plastic containers (35 x 35 mm) were filled with 10% moisture (w/w) level of sieved soil for each nematode

species, concentration and observation period. The nematodes of different concentrations 25, 50, 75, 100 and 200 IJs/pupa in 0.5 ml of D.W. were applied to the container and kept for at least 30 minutes. 1 inscet pupa was placed in each of the container and covered with its lid. Numbers of insect mortality were recorded every 24 hrs till 120 hrs. Pupa placed on wetted filter paper without IJs served as control. 3 replicates (containing 8 insects each) for each nematode species, concentration and observation period were set.

iii) Progeny Production

Progeny production of IJs was determined by the number of IJs produced per larva/pupa (within 20 days), following their exposure to IJs of EPN species at different concentrations. Eight nos. of petridishes were lined with double layer of Whatman No.1 filter paper for each nematode concentration. Nematodes of different concentrations (25, 50, 75, 100 and 200 IJs/larva for *H. chalybaeus* and 10, 25, 50, 75 and 100 IJs/larva for other insect pests larva in 0.5 ml of D. W.) were evenly distributed on filter papers and kept for at least 30 minutes. One insect larva was placed in each of the petriplates and sealed with parafilm.

Assays for pupal mortality were undertaken for *L. orbonalis* and *A. proxima.* Eight nos. of plastic containers (35 x 35 mm) were filled with 10% moisture (w/w) level of sieved soil for each IJs

concentration. The nematodes of different concentrations 25, 50, 75, 100 and 200 IJs/pupa in 0.5 ml of D.W. were applied to the container and kept for at least 30 minutes. 1 pupa was placed in each of the container and covered with its lid.

Larval/pupal mortality was checked at 24 hours interval. The dead larvae/pupae were picked up from each nematode species and concentration. They were rinsed in D.W. and kept in White traps separately to determine the total number of IJs produced in each case. There were 8 replicates for each nematode species and concentration for larvae and pupae separately. To each concentration, one petridish/container, prepared as described above but without IJs was kept as control.

Statistical Analysis:

The data were analyzed statistically and are represented as mean ± standard error of mean (SEM). The significance of the difference was determined by the one way analysis of variance (ANOVA) and student's *t*-test. Probability less than 5% (p values < 0.05) was accepted as statistically significant. Correlation between the parameters was determined by regression analysis. LC_{50} and LT_{50} values were determined and estimated by probit analysis using SPSS software.

Results

A. *Ecological characterization*

1. Effect of temperature on:

i) *Infectivity of IJs:*

Temperature had significant effects on establishment of IJs of *H. indica* on wax moth larvae *($F = 16.606$; $df = 4, 35$; $p < 0.05$).* *H. indica* appeared to be best adapted to temperatures between 15 and 35^{0}C with a more optimum temperature range of 20–30^{0}C. Its establishment was not observed at 10^{0}C (Fig. 3.1 A). Establishment of *S. thermophilum* IJs was significantly affected by temperature *($F = 6.616$; $df = 3, 28$; $p < 0.05$).* IJs did not establish at 10 and 15^{0}C. It appeared to be adapted to temperatures between 20 and 35^{0}C with an optimum temperature of 25–35^{0}C (Fig. 3.1 B). Temperature also had significant affects on establishment of IJs of *S. glaseri* on wax moth larvae *($F = 6.647$; $df = 4, 35$; $p < 0.05$).* The species appeared to be best adapted to temperatures between 15 and 35^{0}C (Fig. 3.1 C).

ii) *Reproduction:*

Temperature had a significant effect on emergence *($F = 19.539$; $df = 3, 28$; $p < 0.05$)* and progeny production *($F = 204.059$; $df = 3, 28$; $p < 0.05$)* of *H. indica* from wax moth larvae (Fig. 3.2 A).

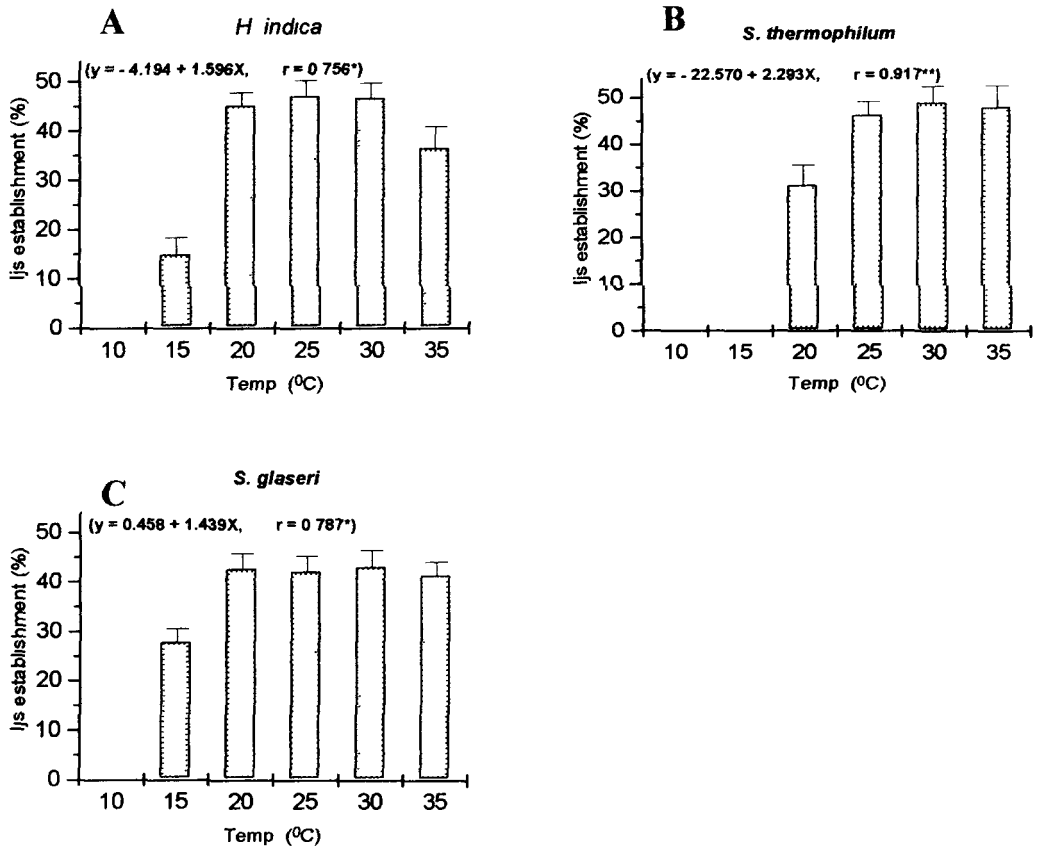


Figure 3.1 A-C: Effect of temperature on establishment of IJs.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
** *Significant at 0.01%* and **significant at 0.05%.*

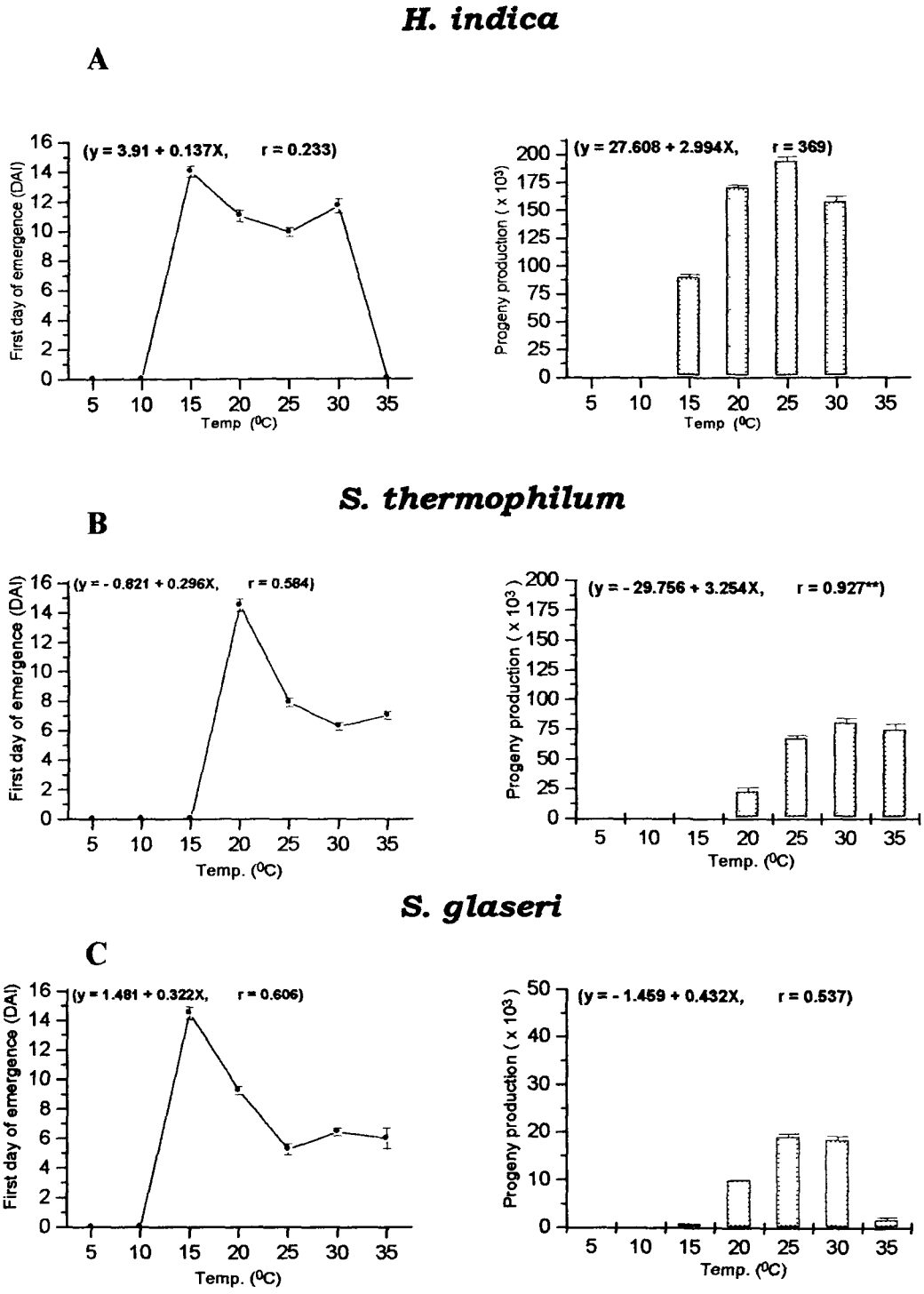


Figure 3.2 A-C: Effect of temperature on emergence and production of IJs. A. *H. indica* B. *S. thermophilum* C. *S. glaseri.*
***Significant at 0.01%.*

No emergence of IJs was observed at 10 and 35^{0}C during the whole observation period (20 days). The IJs could emerge from cadavers only at 15, 20, 25 and 30^{0}C. The earliest emergence of IJs observed was 9.9 ± 0.29 days post inoculation and occurred at 25^{0}C. At 20, 30 and 15^{0}C the IJs emerged at 11.0 ± 0.38, 11.6 ± 0.49 and 14.0 ± 0.38 days after inoculation, respectively. The total number of IJs produced per cadaver varied along with the temperature. Production of IJs was highest at 25^{0}C, yielding 194.2 x 10^3 IJs/cadaver, which was significantly higher than the production of IJs at 20, 30 and 15^{0}C, yielding 170.0 x 10^3, 157.0 x10^3 and 90.0 x 10^3 IJs/cadaver, respectively. As no nematodes emerged at temperatures 35^{0}C and 10^{0}C, no IJs production was recorded within the study period (Plate 3.6).

In *S. thermophilum* the time of first emergence of IJs was affected by temperature *($F = 143.637$; $df = 3, 28$; $p < 0.05$)*. IJs emerged at 14.5 ± 0.42, 7.9 ± 0.29, 6.2 ± 0.25 and 7.0 ± 0.27 days after inoculation at 20, 25, 30 and 35^{0}C, respectively. No emergence was observed at 10 and 15^{0}C. Temperature also played significant role on progeny production *($F = 58.180$; $df = 3, 28$; $p < 0.05$)* with 23.2 x 10^3, 67.8 x 10^3, 80.8 x 10^3 and 75.3 x 10^3 IJs/larva production at 20, 25, 30 and 35^{0}C, respectively (Fig. 3.2 B).

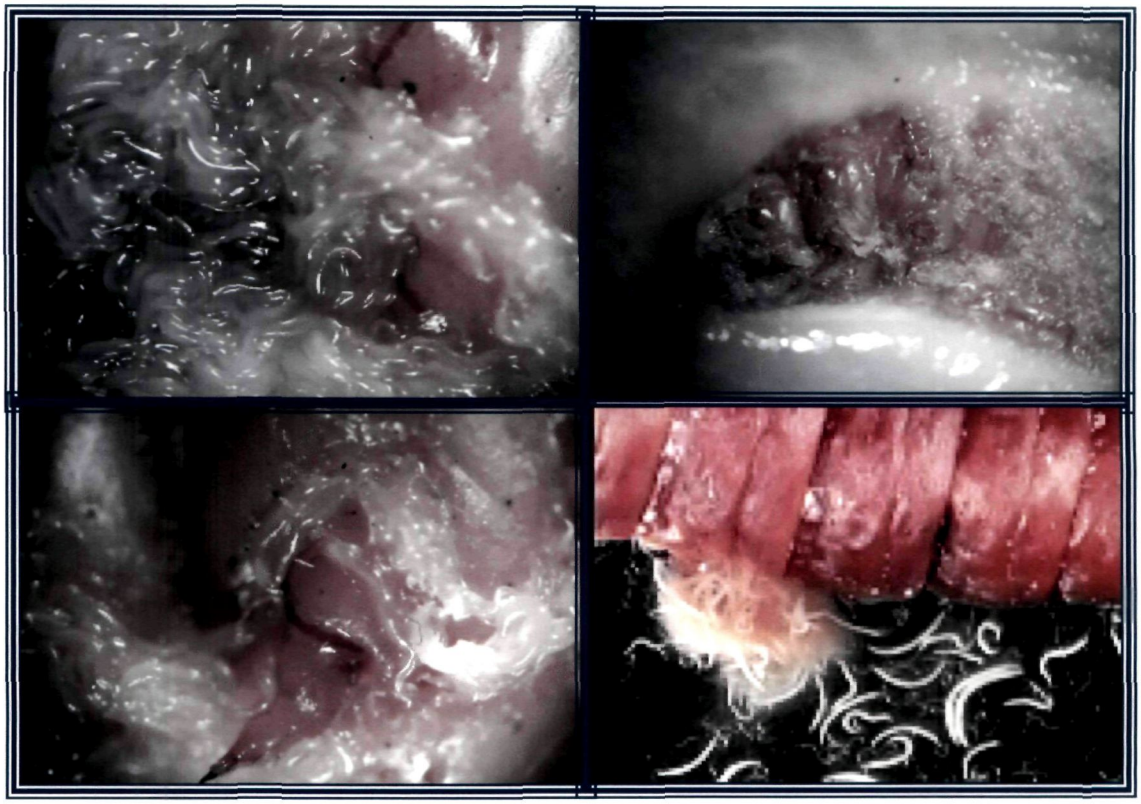


PLATE 3.6: Emergence and production of infective juveniles of *Heterorhabditis* sp. and *Steinernema* spp. from insect cadavers.

In *S. glaseri* the time of first emergence of IJs was affected by temperature *($F = 28.754$; $df = 4, 35$; $p < 0.05$)*. The IJs emerged at 14.5 ± 0.38, 9.3 ± 0.27, 5.3 ± 0.37, 6.4 ± 0.26 and 6.0 ± 0.71 days after inoculation at 15, 20, 25, 30 and 35^{0}C, respectively. No emergence was observed at 10^{0}C. Temperature affected progeny production *($F = 282.520$; $df = 4, 35$; $p < 0.05$)* yielding 0.8 x 10^3, 10.0 x 10^3, 19.1 x 10^3, 18.5 x 10^3 and 1.7 x 10^3 IJs/larva at 15, 20, 25, 30 and 35^{0}C, respectively (Fig. 3.2 C).

2. Effect of relative humidity on reproduction:

In *H. indica* time of first emergence of IJs was affected by relative humidity *($F = 97.269$; $df = 3, 28$; $p < 0.05$)*. The IJs emerged at 16.5 ± 0.19, 11.1 ± 0.29, 10.8 ± 0.45 and 10.0 ± 0.19 days after inoculation at 70, 80, 90 and 100 ± 5% R.H., respectively. Relative humidity played a significant role on progeny production *($F = 609.562$; $df = 3, 28$; $p < 0.05$)* with 21.9 x 10^3, 179.1 x 10^3, 181.9 x 10^3 and 194.2 x 10^3 IJs/larva production at 70, 80, 90 and 100 ± 5% R.H., respectively (Fig. 3.3 A).

The first day of emergence of *S. thermophilum* from wax moth larvae showed less variation with 6.5 ± 0.34, 6.0 ± 0.27, 6.1 ± 0.3 and 6.3 ± 0.24 days after inoculation at 70, 80, 90 and 100 ± 5% R.H., respectively. Progeny production was significantly affected by R.H. *($F = 72.906$; $df = 2, 21$; $p < 0.05$)* with a yield of 7.81 x 10^3, 23.23 x 10^3, 60.7 x 10^3 and 63.2 x 10^3 IJs/larva at 70,

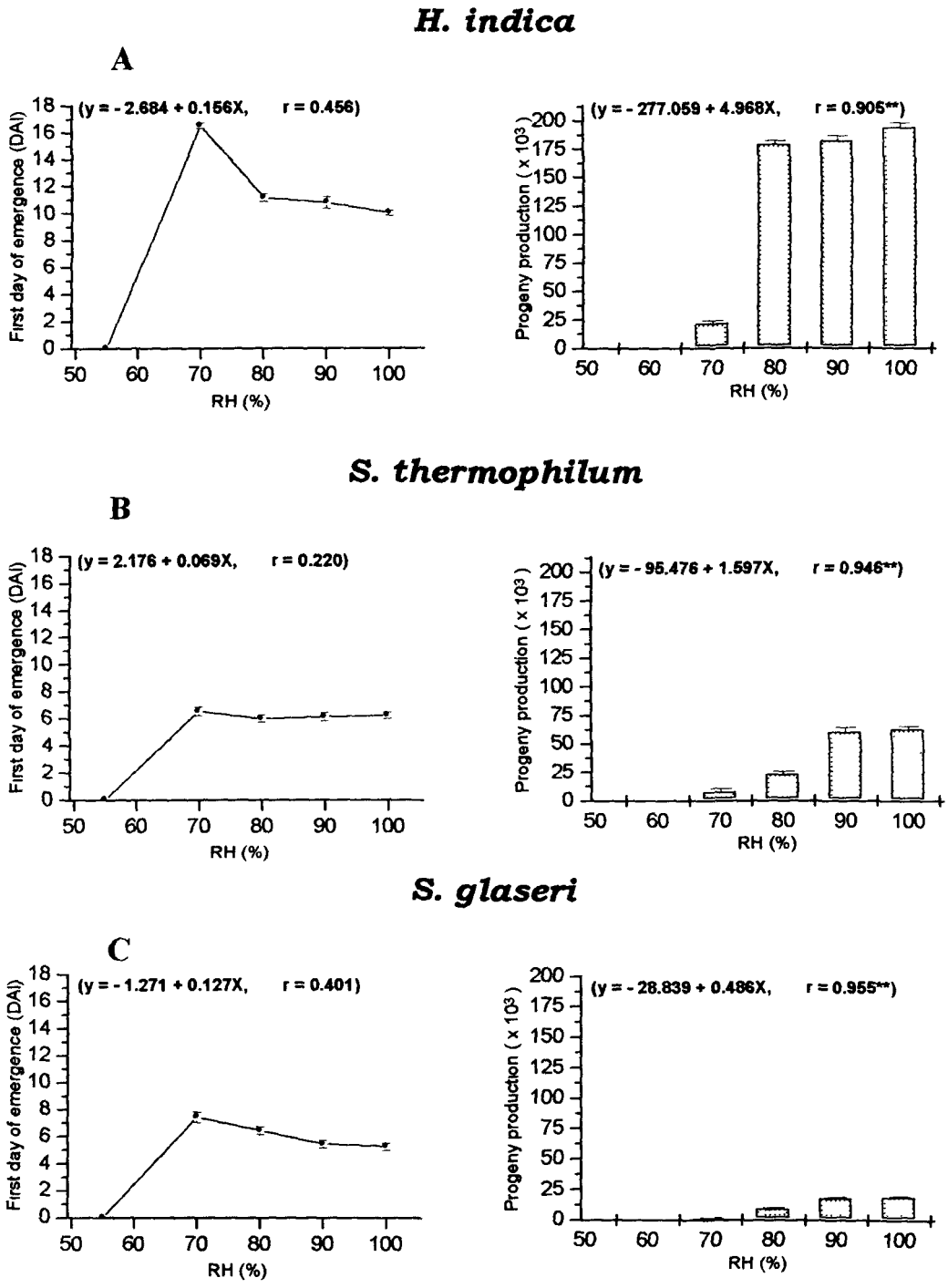


Figure 3.3 A-C: Effect of relative humidity on emergence and production of IJs. A: *H. indica* B. *S. thermophilum* C. *S. glaseri*
***Significant at 0.01%.*

80, 90 and 100 ± 5% RH, respectively (Fig. 3.3 B). In case of *S. glaseri,* R.H. did not show any significant affects on emergence of IJs, whereas the progeny production was significantly affected by R.H. *($F = 155.706$; $df = 3, 25$; $p < 0.05$)* with a yield of 1.6 x 10^3, 9.3 x 10^3, 17.9 x 10^3 and 19.1 x 10^3 IJs/larva at 70, 80, 90 and 100 ± 5% R. H., respectively (Fig. 3.3 C).

3. Storage and persistence of IJs:

At 5^{0}C, survival of IJs was observed till 60 days observation period only in case of *H. indica* and *S. thermophilum* (Fig. 3.4 A; Fig. 3.6 A), higher rate of survival (74 – 86%) were observed within 15 days at all population densities studied; the highest survival rate being observed at 500 IJs/ml in case of *H. indica* while it was 100 IJs/ml in case of *S. thermophilum.* For these two species, survival rate drastically reduced (28–32%) from 30 days observation period. Infectivity was significantly affected by storage duration in *H. indica ($F = 4.166$; $df = 2, 15$; $p < 0.05$)* and *S. thermophilum ($F = 6.098$; $df = 2, 15$; $p< 0.05$).* No significant differences on infectivity were observed between control and IJs survived within 15 and 30 days observation period. However, infectivity was significantly affected within 60 days when compared to control ($p < 0.05$) for both the species. In contrast, IJs of *S. glaseri* survived till 120 days observation period at all population densities, higher rate of survival was observed within

15 days and no significant differences were observed between all population densities studied; the highest mean survival rate being observed at 1000 IJs/ml (99.37%) (Fig. 3.8 A). The survival rate significantly reduced at 120 days observation period at all the population densities. Infectivity was significantly affected by storage time in *S. glaseri* ($F = 4.322$; $df = 4, 35$; $p < 0.05$). No significant differences on infectivity were observed between control and IJs survived within 15, 30 and 60 days observation period. However, infectivity was significantly affected within 90 and 120 days ($p < 0.05$) when compared to control.

At 10^{0}C, for all population densities, survival of IJs was observed till 120 days observation period in case of *H. indica* and *S. glaseri* (Fig. 3.4 B; 3.8 B); however, no IJs of *S. thermophilum* survived at 500 IJs/ml (Fig. 3.6 B). At 100 and 1000 IJs/ml population densities, below 12% IJs survived within 120 days observation period. For all population densities, higher rate of survival (71.6 – 100%) was observed till 60 days observation period; the highest survival rate was observed at 500 IJs/ml in case of *H. indica* and 100 IJs/ml in case of *S. glaseri*. In case of *S. thermophilum*, higher rate of survival (> 80%) was observed within 15 days observation period only. The survival rate of IJs significantly reduced from 90 days observation period for *H. indica*, 60 days observation period for *S. thermophilum* and 120

days observation period for *S. glaseri.* Infectivity was significantly affected by duration in *H. indica (F = 7.302; df = 2, 25; p < 0.05), S. thermophilum (F = 7.635; df = 2, 25; p< 0.05)* and *S. glaseri (F = 4.102; df = 4, 35; p < 0.05).* No significant differences on infectivity were observed between control and IJs survived within 15, 30 and 60 days observation period. However, infectivity was significantly affected within 90 and 120 days ($p < 0.05$) when compared to control for all the species.

At 25^{0}C, survival of IJs was observed till 120 days observation period for all the species at all population densities (Figs. 3.5 A, 3.7 A, 3.9 A). For *H. indica,* highest rate of survival was observed at 100 IJs/ml as compared to other population densities. Significant reductions of survival of IJs were observed at 500 IJs/ml and 1000 IJs/ml at 120 days observation period. In case of *S thermophilum,* higher rate of survival (above 75%) was observed till 60 days at 100 IJs/ml, 30 days at 500 IJs/ml and 15 days at 1000 IJs/ml. In *S glaseri,* higher rate of survival (> 75%) was observed till 60 days at 100 and 500 IJs/ml but till 30 days at 1000 IJs/ml. Infectivity was significantly affected by duration in *H. indica (F = 3.836; df = 4, 25; p < 0.05).* Significant differences in infectivity were observed between control and IJs survived within 90 and 120 days *(p < 0.05)* observation period in case of *H. indica*

and *S. thermophilum,* 120 days *($p < 0.05$)* observation period in case of *S. glaseri.*

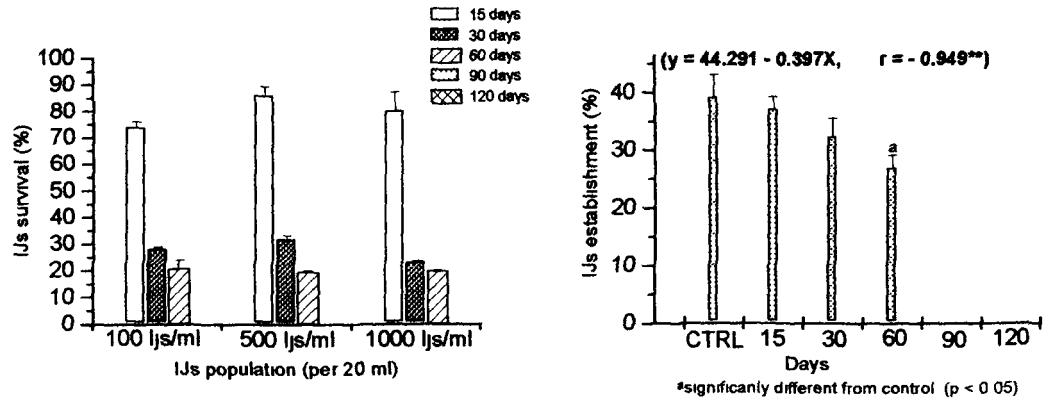
At 30°C, higher rate of survival was observed within 15 days observation period for all three species (Figs. 3.5 B, 3.7 B, 3.9 B). Survival rate significantly reduced from 30 days observation for all the species except *S. thermophilum,* where the survival rate was above 70% within 30 days observation period at 100 IJs/ml population density. Survival was observed till 60 days observation period in case of *S. glaseri* but 90 days for both *H. indica* and *S. thermophilum.* Infectivity was significantly affected by duration in *H. indica ($F = 4.786$; $df = 3, 20$; $p < 0.05$), S. thermophilum ($F = 7.088$; $df = 3, 28$; $p < 0.05$)* and *S. glaseri ($F = 6.011$; $df = 2, 21$; $p < 0.05$).* Significant differences in infectivity of IJs were observed between control and IJs that survived within 30, 60 and 90 days *($p < 0.05$)* observation period in case of *H. indica* and *S. thermophilum,* whereas 30 and 60 days *($p < 0.05$)* observation period only in case of *S. glaseri.*

4. Foraging behavior:

i) *Attachment of IJs to the insect host:*

The attachment of IJs of *H. indica* to a mobile host was higher at all observation time as compared to *S. thermophilum* and *S. glaseri.* The attachment rate was strongly positively correlated with the observation time for all the species.

5°C A



10°C B

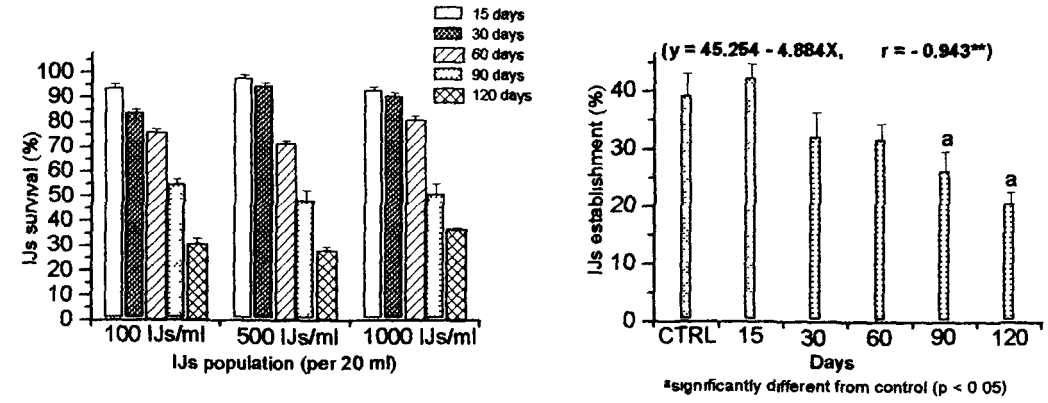


Figure 3.4 A-B: Effect of storage condition on survival and pathogenicity of IJs of *H. indica.*
A: 5°C B: 10°C
***Significant at 0.01%.*
[a] *Significant at 0.05%.*

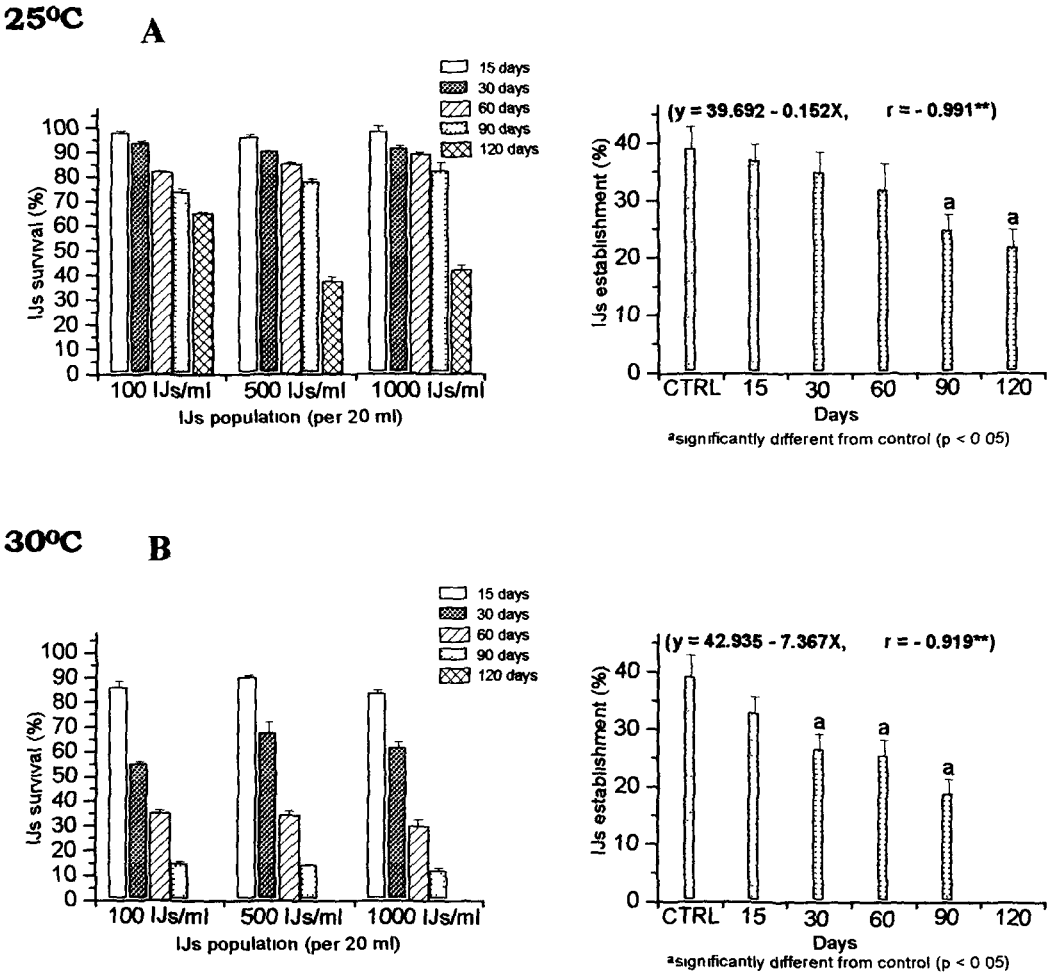


Figure 3.5 A-B: Effect of storage condition on survival and pathogenicity of IJs of *H. indica.*
A: 25°C B: 30°C
***Significant at 0.01%.*
[a] *Significant at 0.05%.*

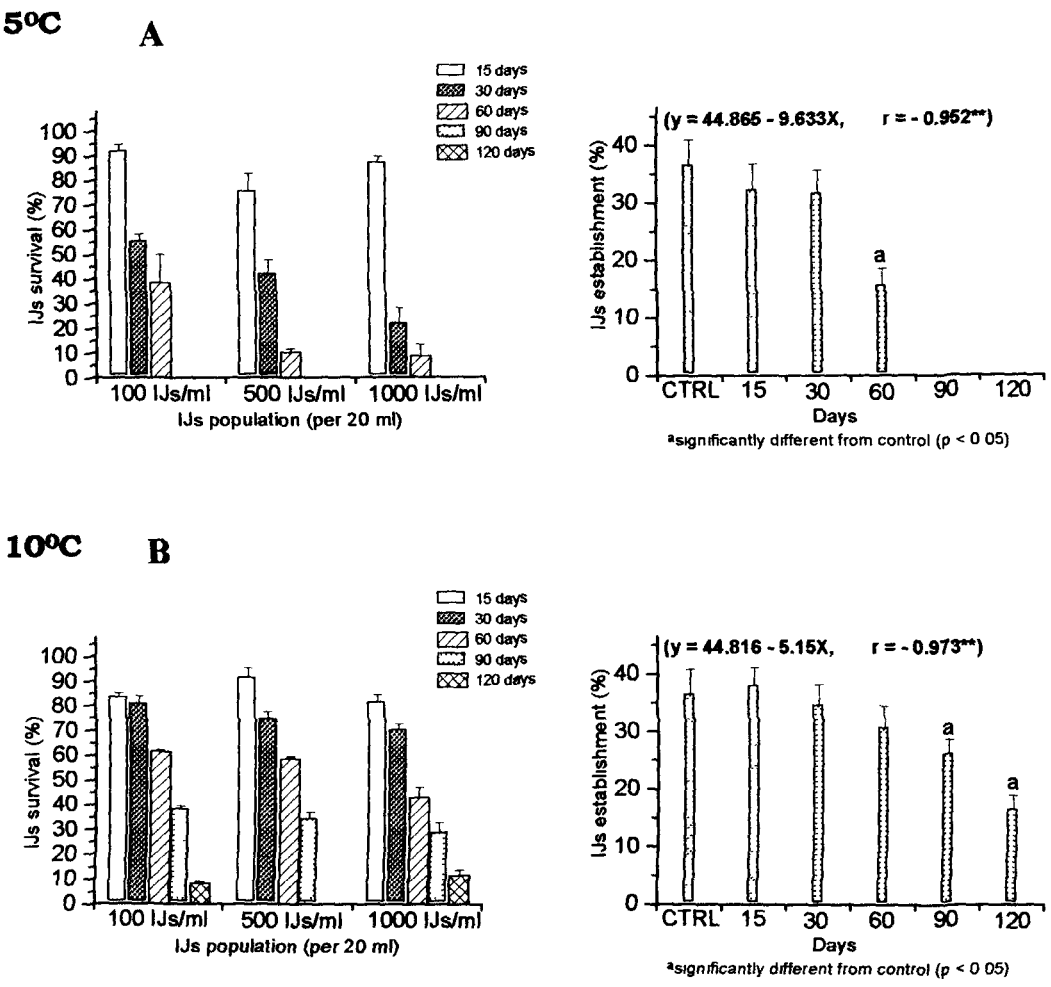


Figure 3.6 A-B: Effect of storage condition on survival and pathogenicity of IJs of *S. thermophilum.*
A: 5°C B: 10°C
** *Significant at 0.01%.*
[a] *Significant at 0.05%.*

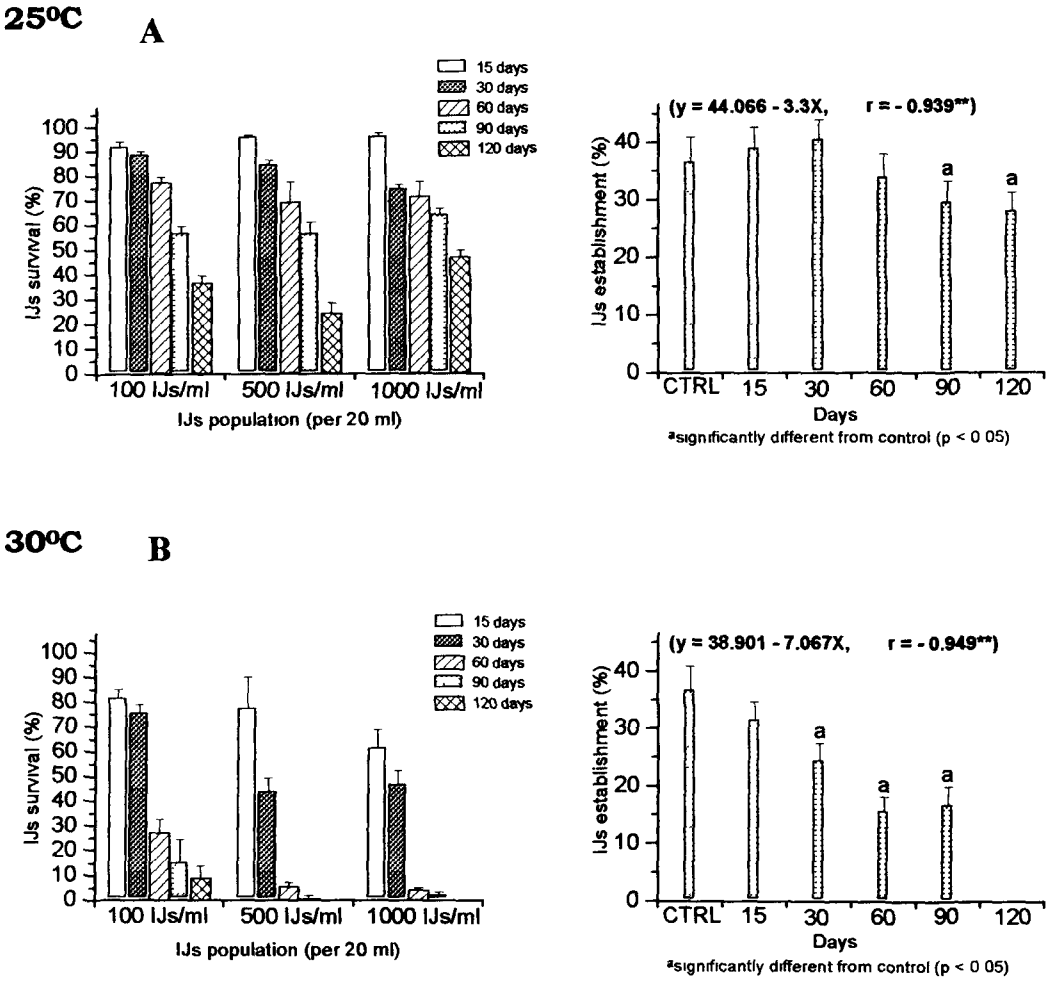
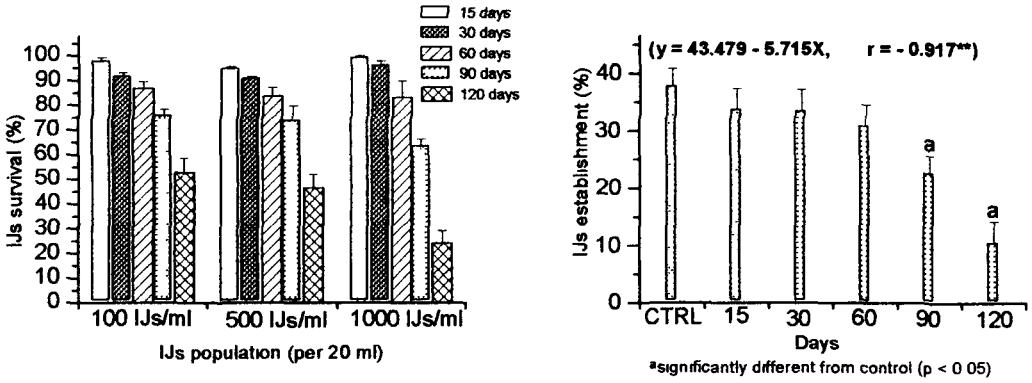


Figure 3.7 A-B: Effect of storage condition on survival and pathogenicity of IJs of *S. thermophilum.*
A: 25°C B: 30°C
***Significant at 0.01%.*
[a] *Significant at 0.05%.*

5°C **A**



10°C **B**

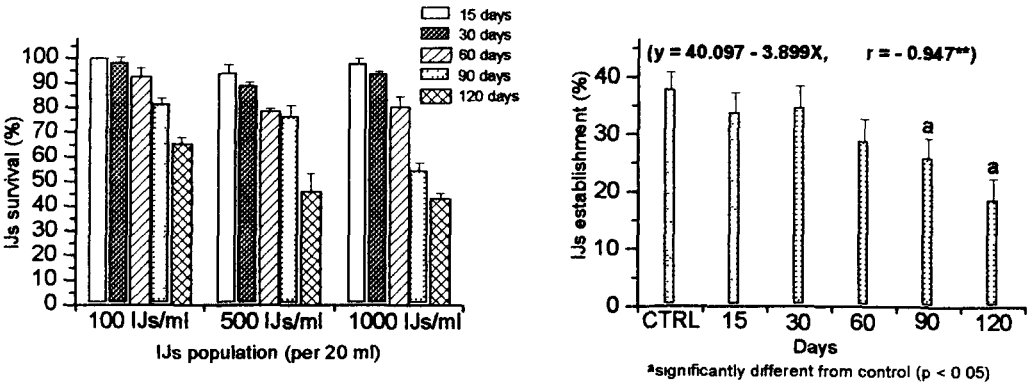


Figure 3.8 A-B: Effect of storage condition on survival and pathogenicity of IJs of *S. glaseri.*
A: 5°C B: 10°C
***Significant at 0.01%.*
[a] *Significant at 0.05%.*

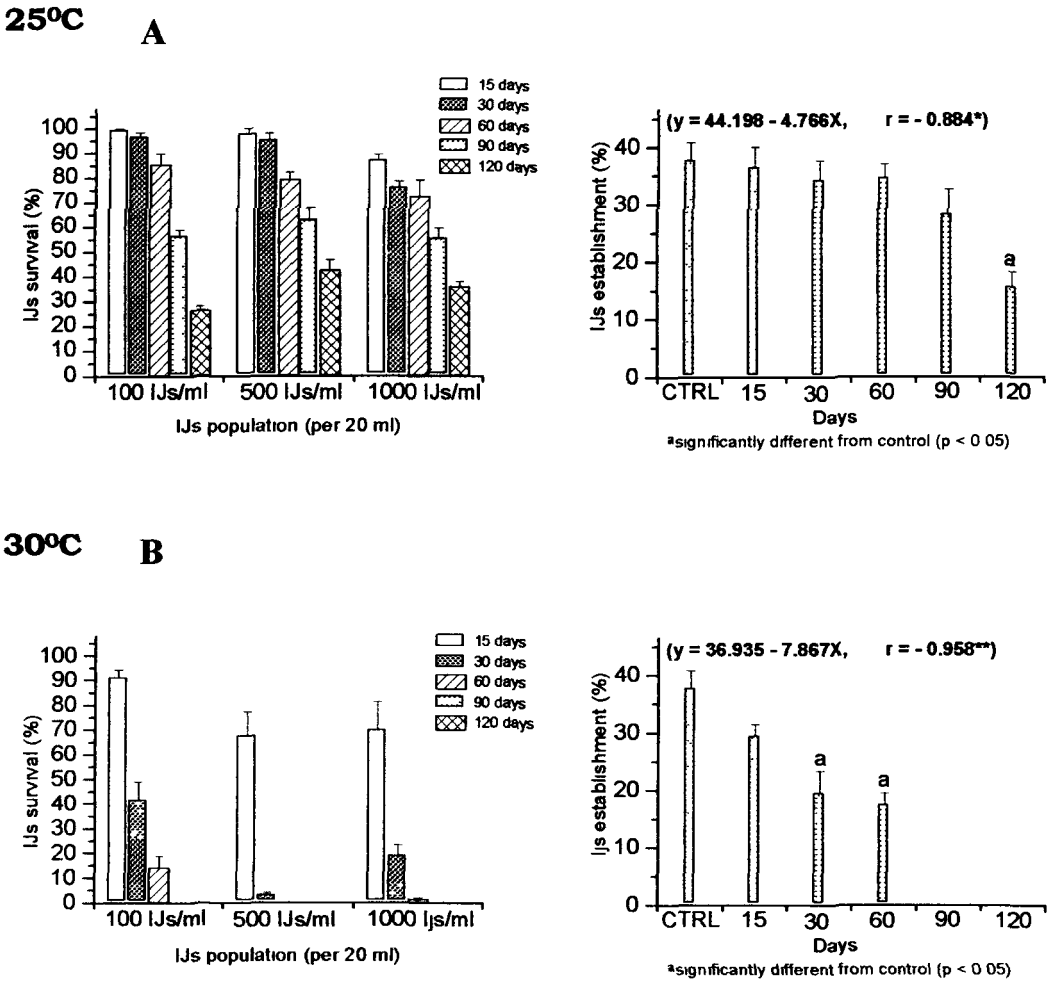


Figure 3.9 A-B: Effect of storage condition on survival and pathogenicity of IJs of *S. glaseri.*
A: 25°C B: 30°C
***Significant at 0.01%.*
[a] *Significant at 0.05%.*

At 1 minute observation time, attachment of IJs of *H. indica* (4.75 ± 0.84) to a mobile host was significantly higher *($p < 0.05$)* as compared to IJs of *S. thermophilum* (2.63 ± 0.93) and *S. glaseri* (1.25 ± 0.83) but no significant differences were observed between *S. thermophilum* and *S. glaseri*. At 5 and 10 minutes observation time, attachment of IJs of *H. indica* (11.24 ± 2.12 and 16.28 ± 2.67) to a mobile host was significantly higher *($p < 0.05$)* as compared to IJs of *S. thermophilum* (6.24 ± 2.12 and 12.47 ± 1.32) and *S. glaseri* (4.12 ± 1.54 and 7.5 ± 1.84). Significant differences *($p < 0.05$)* on attachment of IJs to a mobile host between *S. thermophilum* and *S. glaseri* were also observed at both the observation time (Fig. 3.10 A).

ii) *Effect of soil depth:*

In vertical soil columns, the establishment rate of IJs to the host declined as the soil depth increased (Fig. 3.10 B). The establishment rate of IJs of *H. indica* at 2 cms (65.5%) soil depth was significantly higher *($p < 0.05$)* against 5 cms (35.53%) soil depth. No establishment was observed at 10 cms soil depth. At 2 cms soil depth, 59.93% of IJs of *S. thermophilum* established inside the host cadaver, whereas 52.72% of IJs established at 5 cms soil depth. There was no statistical difference between the two soil depths; however, the establishment rate of IJs at 10 cms

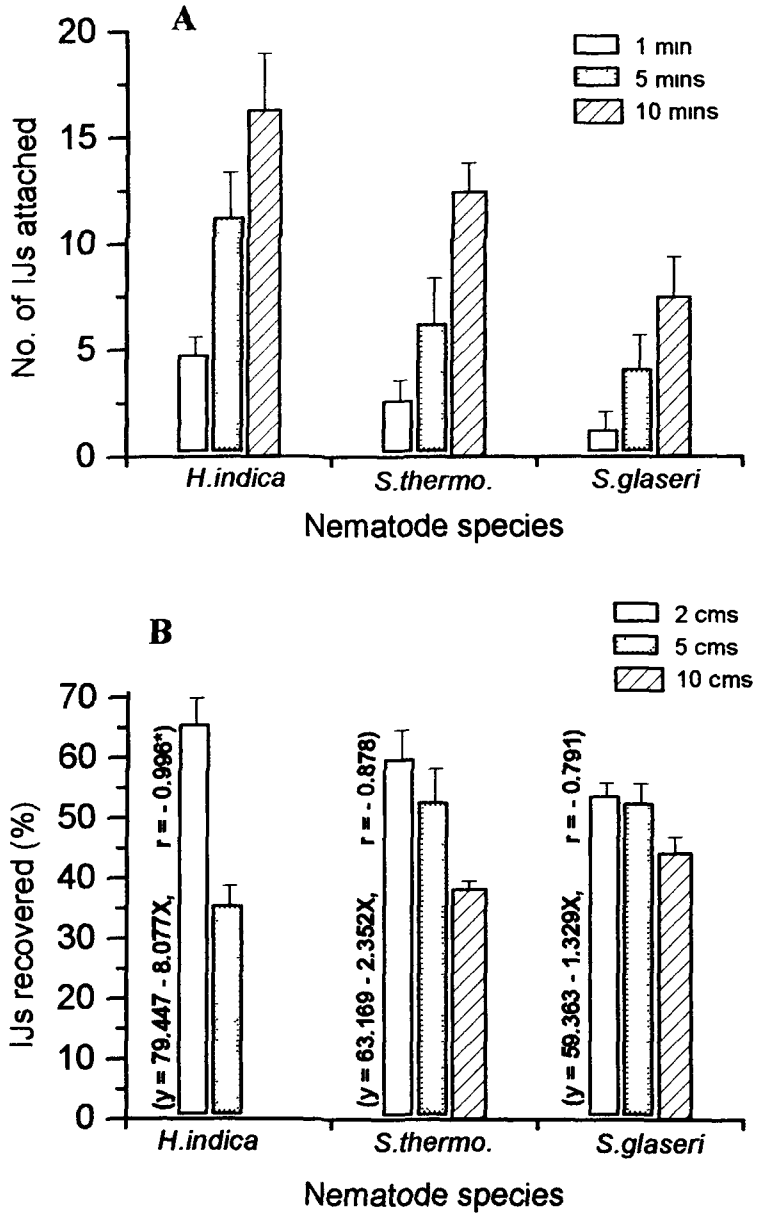


Figure 3.10 A-B: A. Attachment of IJs to insect host B. Effect of soil depth on infectivity.
**Significant at 0.05%.*

(38.35%) was significantly lower *(p < 0.05)* when compared with the other two depths. Similarly, there was no statistical difference on the establishment rate of IJs of *S. glaseri* between 2 cms (53.73%) and 5 cms (52.74%) soil depths; however, the establishment rate of IJs at 10 cms (44.28%) was significantly lower *(p < 0.05)* when compared to 2 and 5 cms soil depths.

5. Soil moisture:

Different soil moisture levels showed significant affects on the establishment of *H. indica (F=19.951, df = 10, 51; p < 0.05)*, except for 8 to 18% soil moisture levels where no significant differences were observed *(p > 0.05)* (Fig. 3.11 A). In general, significant affects of soil moisture levels were also observed in case of *S. thermophilum* (*F = 8.871; df = 10, 51; p* < 0.05) (Fig. 3.11 B) and *S. glaseri* (*F= 8.849; df = 10, 51; p* < 0.05) (Fig. 3.11 C). However, *S. thermophilum* did not show any significant difference from 6 to 20% soil moisture levels. There was an increase in the establishment of IJs as the soil moisture increased but the same showed a declining trend from 20% soil moisture level in case of *H. indica* and *S. thermophilum*. In contrast, establishment of IJs of *S. glaseri* increased along with the soil moisture levels and no decline of establishment was observed even at the highest soil moisture level. Further, it did not show any significant difference between 14 to 25% soil moisture levels (*p* > 0.05).

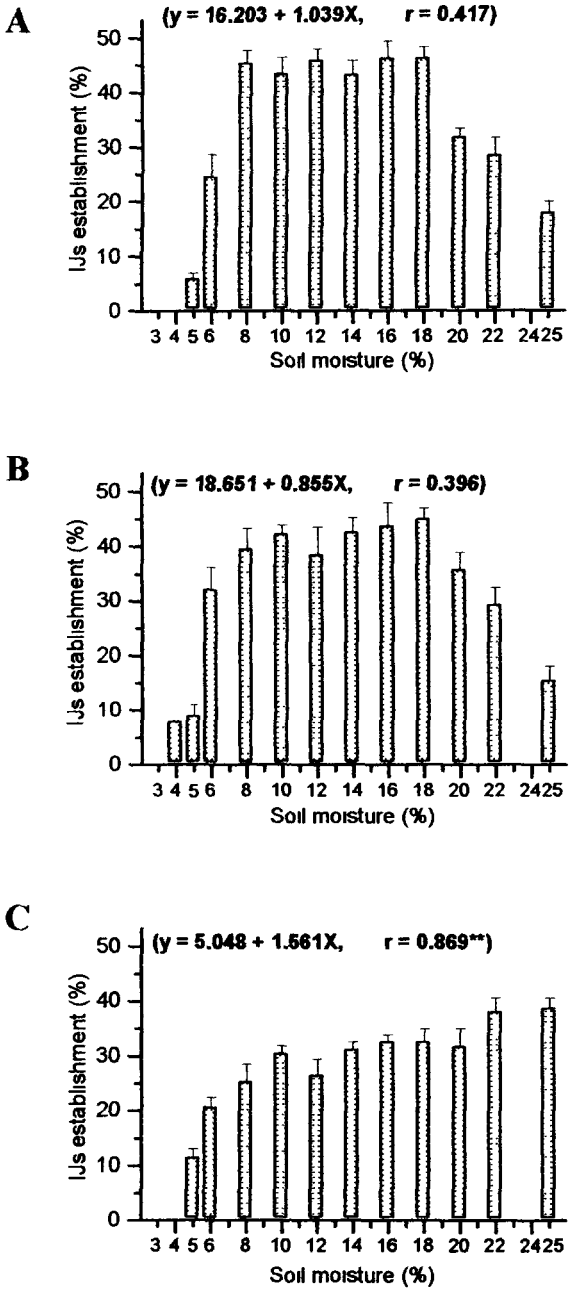


Figure 3.11 A-C: Effect of soil moisture on establishment of IJs.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
***Significant at 0.01%.*

6. Effect of population density on reproduction:

The number of IJs established per insect host significantly increased at higher concentrations for *H. indica (F = 63.656; df = 10, 77; p < 0.05). S. thermophilum (F = 49.692; df = 10, 77; p < 0.05)* and *S. glaseri (F = 179.133; df = 10, 77; p < 0.05).* Establishment of IJs at different concentrations increased from 5.4 ± 0.89 IJs at concentration of 10 IJs/larva to 781 ± 43.1 IJs at 8000 IJs/larva for *H. indica,* 6.1 ± 0.64 IJs at concentration of 10 IJs/larva to 870.0 ± 40.71 IJs at 8000 IJs/larva for *S. thermophilum* and 4.3 ± 0.59 IJs at concentration of 10 IJs/larva to 731.4 ± 29.6 IJs at 8000 IJs/larva for *S. glaseri.* However, the penetration efficiency (percentage of the initial inoculum established in host) decreased linearly with the increase in concentrations for *H. indica (y = 68.009 - 5.730 x, r = - 0.943)* (Fig. 3.12 A), *S. thermophilum (y = 68.888 - 5.604 x, r = - 0.971)* (Fig. 3.13 A) and *S. glaseri (y = 50.081 - 3.446 x, r = - 0.931)* (Fig. 3.14 A). Penetration efficiency of IJs at different concentrations declined from 53.8 ± 8.9% at concentration of 10 IJs/larva to 9.7 ± 0.54% at 8000 IJs/larva for *H. indica,* 61.3 ± 6.4% at concentration of 10 IJs/larva to 10.9 ± 0.51% at 8000 IJs/larva for *S. thermophilum* and 42.5 ± 5.9% at concentration of 10 IJs/larva to 9.1 ± 0.37% at 8000 IJs/larva for *S. glaseri.*

The first day of emergence of IJs from the host cadavers were also affected by the population densities. Earlier emergences were observed at higher densities (Figs. 3.12 B; 3.13 B; 3.14 B). IJs production for the three species increased with increasing initial density upto approximately 200 IJs/larva and then the same declined. No IJs were produced at establishment of IJs higher than 551.5 ± 41.92 per host in case of *H. indica;* 428.4 ± 74.73 per host in case of *S. thermophilum;* 321.4 ± 22.96 per host in case of *S. glaseri.*

The numbers of IJs produced per insect host were significantly affected by concentrations of IJs for *H. indica* ($F = 65.416$; $df = 10, 77$; $p < 0.05$) (Fig. 3.12 C), *S. thermophilum* ($F = 125.835$; $df = 7, 68$; $p < 0.05$) (Fig. 3.13 C) and *S. glaseri* ($F = 43.781$; $df = 6, 45$; $p < 0.05$) (Fig. 3.14 C). The highest number of IJs were produced at a concentration of 200 IJs/larva (IJs establishment = 96.9 ± 5.09 IJs) where the production was 183 ± 6.2 x 10^3 IJs/larva for *H. indica*; 200 IJs/larva (IJs establishment = 86.1 ± 7.85 IJs) the production was 101.8 ± 3.02 x 10^3 IJs/larva for *S. thermophilum*; and 100 IJs/larva (IJs establishment = 31.1 ± 2.84 IJs) where the production was 15.3 ± 1.33 x 10^3 IJs/larva for *S. glaseri.*

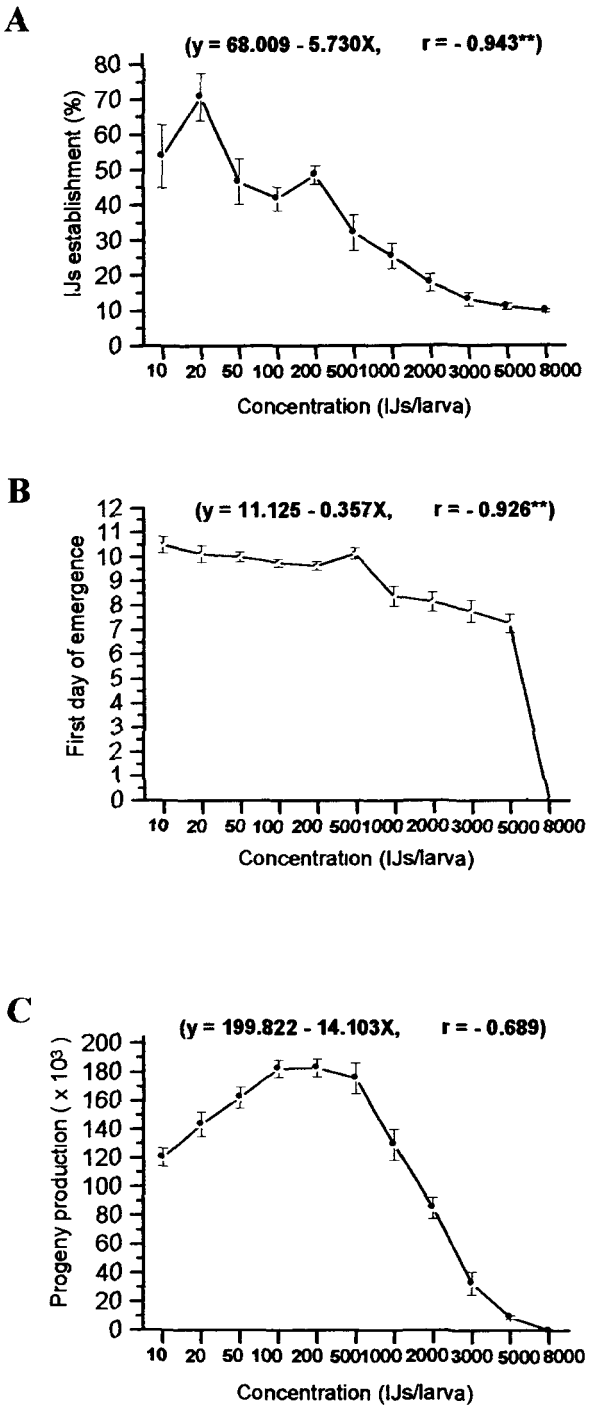


Figure 3.12 A-C: Effect of population densities on *H. indica* IJs:
A. Penetration B. Emergence C. Production
***Significant at 0.01%.*

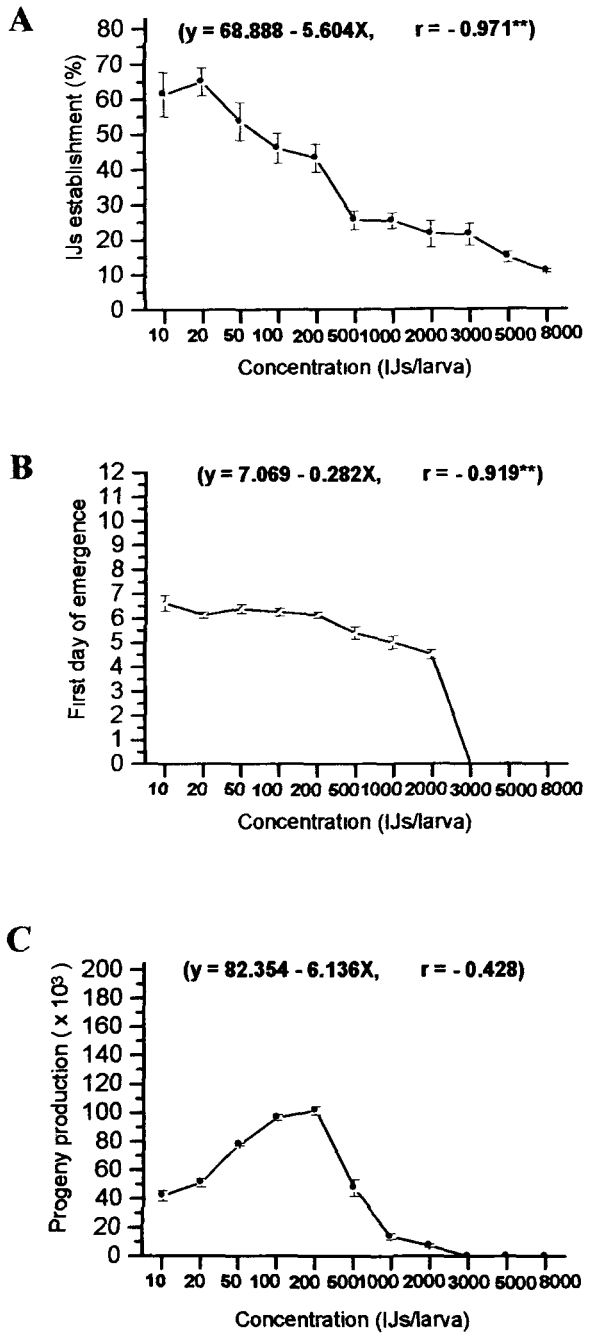


Figure 3.13 A-C: Effect of population densities on *S. thermophilum* IJs.
A. Penetration B. Emergence C. Production
***Significant at 0.01%.*

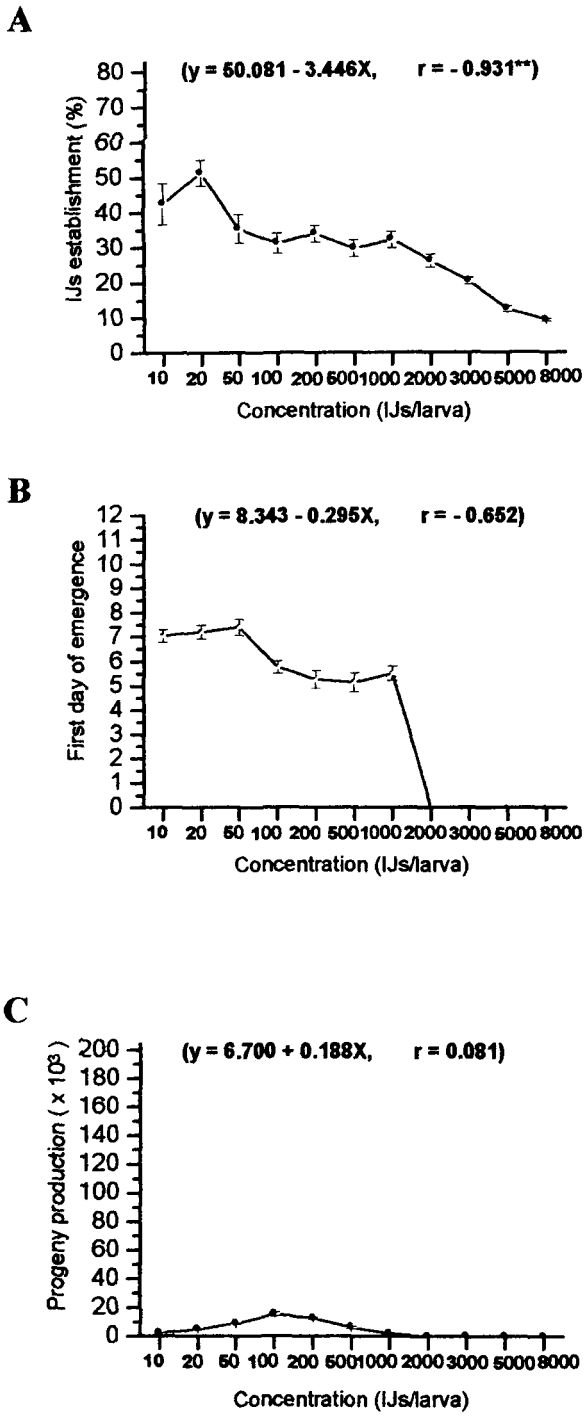


Figure 3.14 A-C: Effect of population densities on *S. glaseri* IJs.
A. Penetration B. Emergence C. Production
**Significant at 0.01%.*

7. Effect of chemical pesticides on survival and pathogenicity of IJs:

No significant differences were observed on survival of IJs of *H. indica* when exposed to mancozeb and *nimbecidine* at both lower and higher concentrations (Fig. 3.15 A). In case of IJs of *S. thermophilum,* no significant differences were observed when exposed to both carbaryl and nimbecidine at lower and higher concentrations, and to mancozeb at lower concentration (Fig. 3.15 B). However, IJs of *S. glaseri* showed a higher rate of survival when exposed to nimbecidine, endosulfan, quinolphos, fenvalerate and mancozeb (Fig. 3.15 C).

Exposure to lower and higher concentration of carbaryl resulted in 64.3 ± 2.17 and 64.5 ± 2.85% survival of IJs of *H. indica*, significantly lower *($p < 0.05$)* than control (97.8 ± 0.9%). Very low survival rate (< 40%) was observed for IJs of *S. glaseri* when exposed to carbaryl at both concentrations. The infectivity of IJs of *H. indica* after exposure to lower (30.5 ± 2.81%) and higher (27.8 ± 2.03%) concentrations was also significantly lower *($p < 0.05$)* than control (38.8 ± 3.56%). Though, there was no significant difference noticed on survival of IJs of *S. thermophilum* exposed to carbaryl as compared to control; however, the infectivity of *S. thermophilum* IJs was significantly low at lower (35.4 ± 2.85%) and higher (36.8 ± 2.68%) concentration than

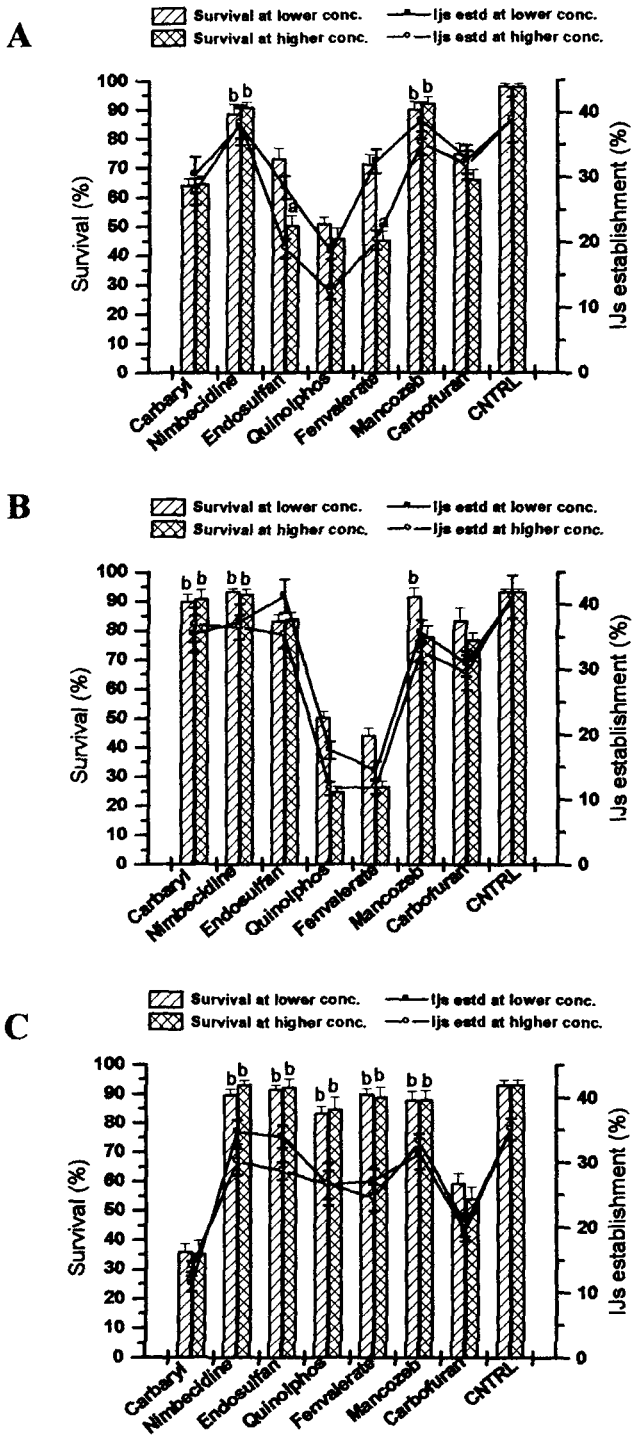


Figure 3.15 A-C: Effect of pesticides on survival and pathogenicity of IJs.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
[b] *Not significant from control.*

control (41.2 ± 3.28%). Carbaryl also had a significant affect *(p < 0.05)* on the infectivity of IJs of *S. glaseri* at lower (11.2 ± 1.18%) and higher (13.9 ± 0.83%) concentrations.

Nimbecidine did not show any effects on survival and infectivity of *H. indica* and *S. thermophilum;* however, exposure of *S. glaseri* IJs to nimbecidine higher concentration showed a significantly lower *(p < 0.05)* infectivity (30.0 ± 2.05%) compared to control (35.1 ± 1.66%).

Exposure to lower and higher concentrations of endosulfan resulted in 73.3 ± 3.39 and 50.4 ± 3.36% survival for IJs of *H. indica,* significantly lower *(p < 0.05)* than control (97.8 ± 0.9%); however, the same showed 83.5 ± 2.09 and 84.0 ± 2.15% survival for IJs of *S. thermophilum,* significantly lower *(p < 0.05)* than control (93.3 ± 0.94%). In contrast, no significant differences were observed between survival of IJs of *S. glaseri* at both concentrations (91.7 ± 1.15 (lower) and 92.3 ± 2.61% (higher) and control (93.3 ± 1.39%). The infectivity of IJs of *H. indica* after exposure to lower (28.5 ± 1.76%) and higher (19.1 ± 1.52%) concentrations were significantly lower *(p < 0.05* and *p < 0.01,* respectively*)* than control (38.9 ± 3.56%). Infectivity of IJs of *S. thermophilum* at lower concentration (41.4 ± 2.56%) did not show any significant difference except that it was significantly lower *(p < 0.05)* at higher (35.4 ± 2.03%) concentration than control (41.3 ±

3.28%). The infectivity of IJs of *S. glaseri* at endosulfan's lower concentration (33.9 ± 1.71%) did not reveal any significant difference from control. However, the IJs infectivity was significantly lower *($p < 0.05$)* at higher (28.6 ± 1.38%) concentration than control (35.1 ± 1.66%).

Exposure to lower and higher concentrations of quinolphos resulted in 50.9 ± 2.25 and 45.8 ± 3.54% survival for IJs of *H. indica* which was significantly lower *($p < 0.01$)* than control (97.8 ± 0.9%); while IJs of *S. thermophilum* showed 50.4 ± 1.95 and 24.8 ± 1.72% survival which was significantly lower *($p < 0.01$)* than control (93.3 ± 0.94%). In contrast, no significant differences were observed between survival of IJs of *S. glaseri* at both concentrations (83.5 ± 2.09 (lower) and 84.8 ± 3.88% (higher) as compared to control (93.3 ± 1.39%). The infectivity of IJs of *H. indica* after exposure to lower (18.5 ± 1.13%) and higher (12.2 ± 1.03%) concentrations was significantly lower *($p < 0.01$)* than control (38.9 ± 3.56%). Similarly, infectivity of IJs of *S. thermophilum* at lower (17.5 ± 1.32%) and higher (11.6 ± 1.03%) concentrations was significantly lower *($p < 0.01$)* than control (41.2 ± 3.28%). Quinolphos had the same effects on the infectivity of IJs of *S. glaseri* showing significant difference *($p < 0.05$)* between both concentrations and control.

Exposure to lower and higher concentrations of fenvalerate resulted in 71.2 ± 3.28 and 45.3 ± 3.13% survival for IJs of *H. indica,* significantly lower *(p < 0.05* and *0.01,* respectively*)* than control (97.9 ± 0.90%), significant difference *(p < 0.05)* was also observed between the two concentrations. In case of *S. thermophilum,* 44.3 ± 2.58 and 26.7 ± 1.98% survival was observed and it was significantly lower *(p < 0.01)* than control (93.3 ± 0.94%), significant difference *(p < 0.05)* was also observed between the two concentrations. In contrast, no significant differences were observed between survival of IJs of *S. glaseri* at both concentrations (90.2 ± 1.55 (lower) and 89.1 ± 3.33% (higher) and control (93.3 ± 1.39%). The infectivity of IJs of *H. indica* after exposure to lower (32.5 ± 1.83%) and higher (20.4 ± 1.48%) concentrations was significantly lower *(p < 0.05* and *p < 0.01* respectively*)* than control (38.4 ± 3.56%). Interestingly, fenvalerate revealed a serious effect on the infectivity of IJs of *S. thermophilum,* infectivity at lower (14.5 ± 1.32%) and higher (11.9 ± 1.03%) concentrations was significantly lower *(p < 0.01)* than control (41.3 ± 3.28%). In case of *S. glaseri,* though, fenvalerate did not show any significant effects on survival, the infectivity of its IJs at lower (27.1 ± 1.84%) and higher (24.5 ± 2.03%) concentrations was significantly lower *(p < 0.05)* than control (35.1 ± 1.66%).

Mancozeb was found compatible at both concentrations with all the species except for IJs of *S. thermophilum* at higher concentration, where the survival rate was 83.7 ± 3.54% and infectivity rate was 28.7 ± 2.58%, significantly different *($p < 0.05$)* from control.

Exposure to lower and higher concentrations of carbofuran resulted in 83.4 ± 4.39 and 76.8 ± 2.25% survival for IJs of *H. indica,* significantly lower *($p < 0.05$)* than control (97.8 ± 0.9%). In case of *S. thermophilum,* 75.4 ± 3.09 and 65.3 ± 3.13% survival was observed that was significantly lower *($p < 0.05$)* than control (93.3 ± 0.94%); significant difference *($p < 0.05$)* was also observed between the two concentrations. Low survival rate was observed for IJs of *S. glaseri* at both concentrations and were significantly different *($p < 0.01$)* from control. The infectivity of IJs of *H. indica* after exposure to lower (32.7 ± 2.2%) and higher (31.8 ± 2.19%) concentrations was significantly lower *($p < 0.05$)* than control (38.8 ± 3.56%). Even in case of IJs of *S. thermophilum,* infectivity at lower (30.8 ± 1.95%) and higher (29.5 ± 2.68%) concentrations was significantly lower *($p < 0.05$)* than control (41.2 ± 3.28%). Carbofuran had serious effects on IJs of *S. glaseri,* the infectivity of its IJs at lower (19.5 ± 1.59%) and higher (20.3 ± 1.74%) concentrations was significantly lower *($p < 0.05$)* than control (35.1 ± 1.66%).

B. Bioefficacy of EPNs *against insect pests:*

a) *Haplosonyx chalybaeus*

Larval mortality

The results of percent larval mortality caused by three EPN species are presented in (Figs. 3.16 A-i, 3.16 B-i, and 3.16 C-i). The concentrations of IJs were found to be positively correlated with the time of larval mortality (Table 3.1). It may be noted that at 25 IJs/larva, *H. indica* caused mortality from 72 hours after inoculation (HAI) and the same reached to 29.1 ± 4.13% within 120 HAI. This was comparatively higher from larval mortality caused by *S. thermophilum* and *S. glaseri,* where the mortality was first observed at 96 HAI and it was observed to be 20.8 ± 4.13 and 25.0 ± 7.25%, respectively within 120 HAI.

At 50 IJs/larva, mortality caused by both *H. indica* (25.0 ± 7.25%) and *S. glaseri* (16.6 ± 4.13%) was first observed at 48 HAI and reached to 50.0 ± 7.25% within 120 HAI. In contrast, *S. thermophilum* caused mortality from 72 HAI and it was recorded to be 45.8 ± 11.00% within 120 HAI. At 75 IJs/larva, mortality was first observed at 48 HAI for all three EPNs. However, *S. glaseri* caused 100% mortality within 96 HAI. *H. indica* caused only 66.6 ± 8.38% of mortality and *S. thermophilum* caused 79.1 ± 11.00% mortality within 120 HAI.

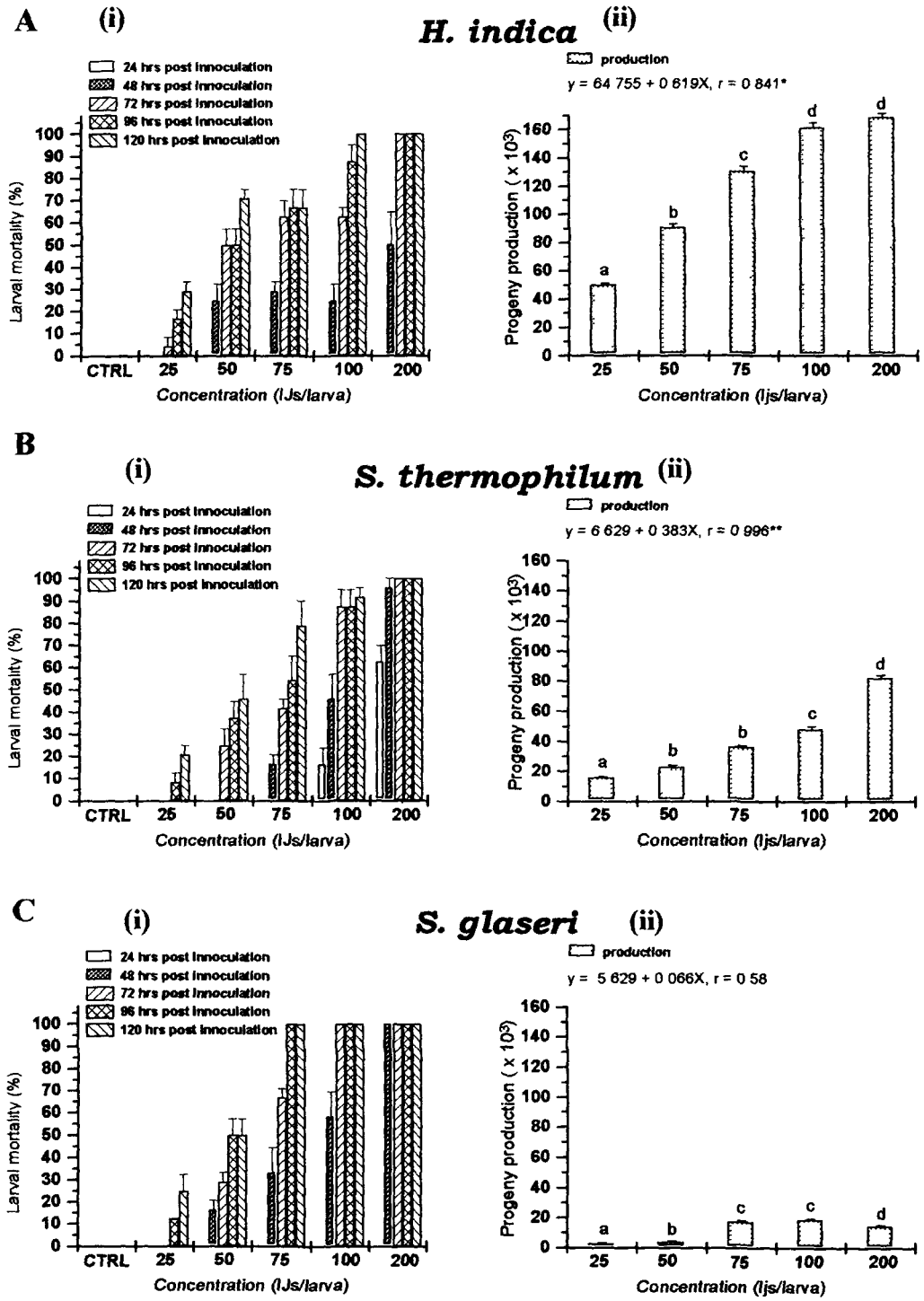


Figure 3.16 A-C: Bioefficacy of EPNs against ***H. chalybaeus*** larvae.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
(i) Larval mortality (ii) Progeny production
***Significant at 0.01%* and **Significant at 0.05%.*
Means shown by the same letter are not significantly different ($p < 0.05$).

Table 3.1: Correlations between the concentrations of EPNs and larval mortality time of *H. chalybaeus*.

Concentrations (IJs/larva)	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
25	0.93*	0.86*	0.88*
50	0.96**	0.98**	0.97**
75	0.91*	0.99**	0.97**
100	0.96**	0.91*	0.87*
200	0.88*	0.76	0.70

Time (hrs)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	0.96**	-
48	0.89*	0.98**	0.98**
72	0.90*	0.90*	0.82*
96	0.88*	0.89*	0.71
120	0.79	0.84*	0.72

***significant at 0.01%* and **significant at 0.05%.*

Table 3.2: LC_{50} and LT_{50} values of EPNs against *H. chalybaeus* larva.

Hours after inoculation	LC_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	**178.0**	-
48	**186.0**	**115.0**	**90.3**
72	**70.1**	**74.4**	**63.4**
96	**56.4**	**65.9**	**46.2**
120	**48.7**	**52.6**	**42.7**

Concentrations (IJs/larva)	LT_{50} (hrs)		
	H. indica	*S. thermophilum*	*S. glaseri*
25	**137.3**	**142.1**	**137.6**
50	**100.4**	**115.4**	**107.2**
75	**80.5**	**88.0**	**60.0**
100	**66.3**	**51.3**	**46.7**
200	**57.1**	**18.8**	**39.0**

At 100 IJs/larva, mortality was first observed at 48 HAI for *H. indica* and *S. glaseri,* whereas the same recorded to be after 24 HAI in case of *S. thermophilum.* At the same concentration, 100% mortality was observed within 72 and 120 HAI in case of *S. glaseri* and *H. indica,* respectively, whereas only 87.5 ± 7.25% mortality was observed within 120 HAI in case of *S. thermophilum.* At 200 IJs/larva, *H. Indica* and *S. glaseri* did not show any mortality at 24 HAI; however, *S. thermophilum* caused 62.5 ± 7.25% mortality within 24 HAI. At the same concentration, 100% larval mortality was observed for all the species, however, the time taken for same varied: 72 HAI in case of both *H. indica* and *S. thermophilum,* and 48 HAI for *S. glaseri.*

No larval mortality was observed in the control groups. The calculated values of LC_{50} and LT_{50} are presented in Table 3.2.

Progeny production:

The progeny production by insect larva was determined by recording the total number of IJs produced per insect larva in a limited time period. The production of IJs increased linearly with the increase in concentrations for *H. indica (y = 64.755 + 0.619 x, r = 0.841)* (Fig. 3.16 A-ii), *S. thermophilum (y = 6.629 + 0.383 x, r = 0.996)* (Fig. 3.16 B-ii) and *S. glaseri (y = 5.629 + 0.066 x, r = 0.581)* (Fig. 3.16 C-ii). The production of IJs at different concentrations increased from 50.4 ± 1.15 x 10^3 IJs/larva at 25 IJs/larva

concentration to 168.9 ± 2.67 x 10^3 IJs/larva at 200 IJs/larva for *H. indica*. For *S. thermophilum*, it was 16.1 ± 0.4 x 10^3 IJs/l at 25 IJs/larva concentration to 82.0 ± 2.07 x 10^3 IJs/larva at 200 IJs/larva. For *S. glaseri* it was 2.8 ± 0.32 x 10^3 IJs/larva at 25 IJs/larva concentration and reached to its maximum of 18.9 ± 0.57 x 10^3 IJs/larva at 100 IJs/larva, and then declined to 14.7 ± 0.59 x 10^3 IJs/larva at 200 IJs/larva concentration.

b) *Pieris brassicae*

Larval mortality

The concentrations of IJs were found to be positively correlated with the time of larval mortality (Table 3.3). At 10 IJs/larva, *H. indica* caused a mortality of 12.5 ± 4.13% from 48 hours after inoculation (HAI) and the same reached to 95.8 ± 4.13% within 120 HAI (Fig. 3.17 A-i). The mortality by *S. thermophilum* and *S. glaseri* was first observed at 48 HAI and it reached up to 95.8 ± 4.13% in case of *S. thermophilum* and 91.6 ± 8.5% for S. *glaseri* within 120 HAI (Fig. 3.17 B-i) (Fig. 3.17 C-i). At 25 IJs/larva, mortality was first observed at 48 HAI for all the three species; *H. indica* (37.5 ± 14.38%), *S. thermophilum* (37.5 ± 7.25%) and *S. glaseri* (20.8 ± 8.5%). Within 120 HAI, mortality reached upto 100% in case of both *H. indica* and *S. thermophilum,* whereas up to 95.8 ± 4.13% in case of *S. glaseri.*

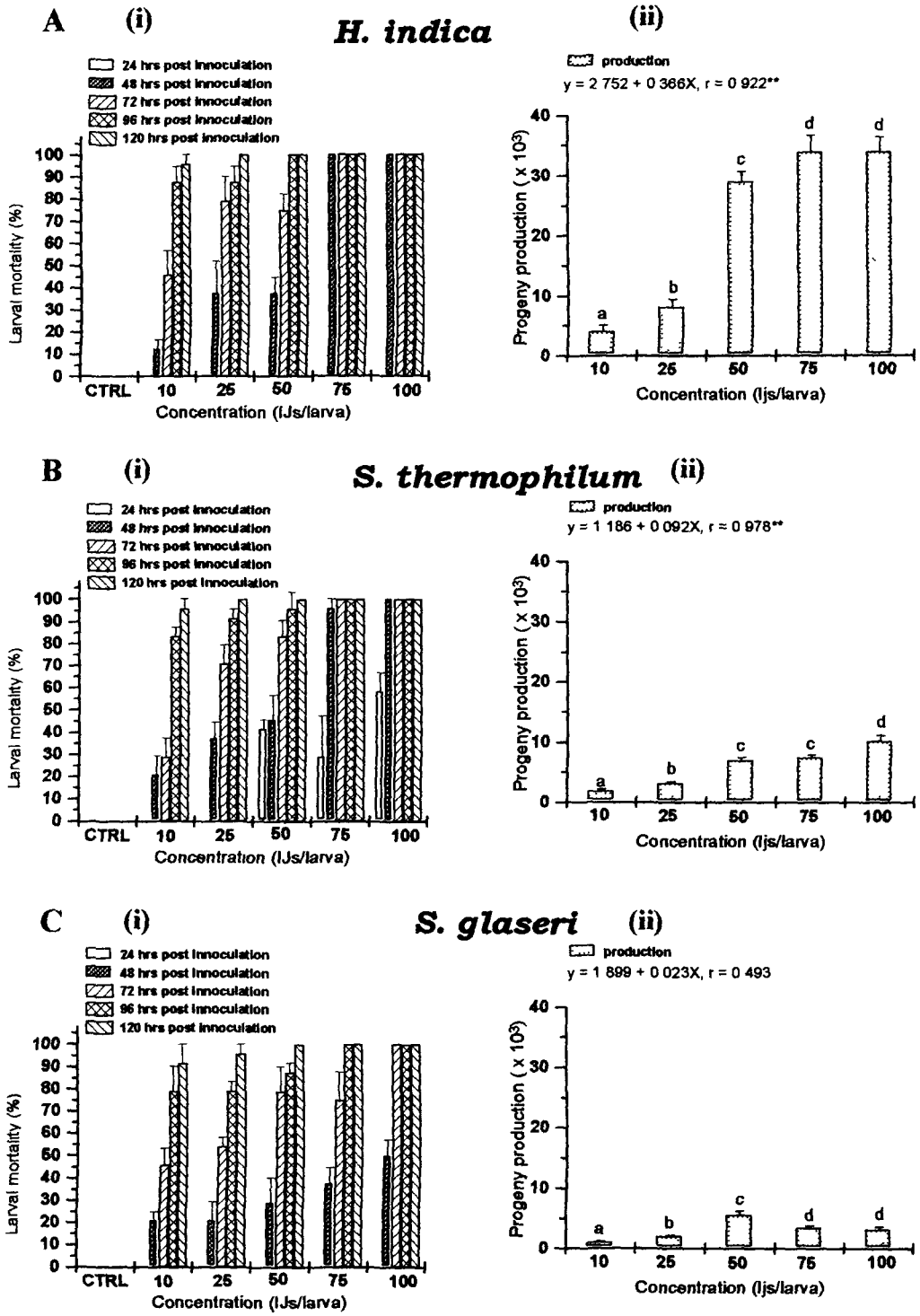


Figure 3.17 A-C: Bioefficacy of EPNs against ***P. brassicae*** larvae.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
(i) Larval mortality (ii) Progeny production
***Significant at 0.01%* and **Significant at 0.05%.*
Means shown by the same letter are not significantly different ($p < 0.05$).

Table 3.3: Correlations between the concentrations of EPNs and larval mortality time of *P. brassicae.*

Concentrations (IJs/larva)	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
10	0.97**	0.96**	0.99**
25	0.95**	0.97**	0.99**
50	0.95**	0.95**	0.95**
75	0.70	0.73	0.95**
100	0.70	0.70	0.88*

Time (hrs)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	0.90*	-
48	0.93**	0.96**	0.97**
72	0.88*	0.88*	0.95**
96	86*	0.91*	0.95**
120	0.64	0.64	0.84

***significant at 0.01%* and **significant at 0.05%.*

Table 3.4: LC_{50} and LT_{50} values of EPNs against *P. brassicae* larva.

Hours after inoculation	LC_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	**87.5**	-
48	**41.1**	**30.2**	**104.9**
72	**10.0**	**17.3**	**17.6**
96	-	-	-
120	-	-	-

Concentrations (IJs/larva)	LT_{50} (hrs)		
	H. indica	*S. thermophilum*	*S. glaseri*
10	**74.3**	**76.8**	**75.4**
25	**59.7**	**60.4**	**72.4**
50	**57.1**	**28.5**	**58.1**
75	**39.0**	**29.7**	**57.1**
100	**39.0**	-	**47.9**

At 50 IJs/larva, mortality was first observed at 48 HAI in case of *H. indica* and *S. glaseri,* whereas it was observed at 24 HAI for *S. thermophilum.* At this concentration, 100% mortality was observed within 96 HAI for *H. indica,* whereas *S. glaseri* and *S. thermophilum* showed 100% mortality at 120 HAI. At 75 IJs/larva, *H. indica* did not cause any mortality within 24 HAI but 100% mortality was observed at 48 HAI. Mortality started at 24 HAI in case of *S. thermophilum,* and it reached to 100% within 72 HAI. *S. glaseri* also caused a 100% larval mortality within 72 HAI.

At 100 IJs/larva, *H. indica* and *S. glaseri* did not cause any larval mortality at 24 HAI; however, *S. thermophilum* showed 58.3 ± 8.5% mortality at 24 HAI. 100% larval mortality was observed at 48 HAI for both *H. indica* and *S. thermophilum* whereas, *S. glaseri* caused 100% mortality at 72 HAI.

No larval mortality was observed in the control groups. The calculated values of LC_{50} and LT_{50} are presented in Table 3.4.

Progeny production:

The production of IJs was positively correlated with the concentrations for *H. indica (y = 2.752 + 0.366 x, r = 0.922)* (Fig. 3.17 A-ii), *S. thermophilum (y = y = 1.186 + 0.092 x, r = 0.978)* (Fig. 3.17 B-ii) and *S. glaseri (y = 1.899 + 0.023 x, r = 0.493)* (Fig. 3.17 C-ii). The production of IJs at different concentrations increased from 4.1 ± 0.946 x 10^3 IJs/larva at 10 IJs/larva concentration to

33.8 ± 2.46 x 10^3 IJs/larva at 100 IJs/larva for *H. indica;* for *S. thermophilum* IJs production varied from 1.8 ± 0.31 x 10^3 IJs/larva at 10 IJs/larva concentration to 10.2 ± 0.85 x 10^3 IJs/larva at 100 IJs/larva concentration. In case of *S. glaseri,* it was 1.1 ± 0.11 x 10^3 IJs/larva at 10 IJs/larva concentration and reached to a highest production of 5.6 ± 0.58 x 10^3 IJs/larva at 50 IJs/larva concentration.

c) ***Leucinodes orbonalis***

Larval mortality

The concentrations of IJs were found to be positively correlated with the time of larval mortality of *L. orbonalis* (Table 3.5). At 10 IJs/larva, the first mortality was observed at 72 HAI for *H. indica* (29.1 ± 11.00%), *S. thermophilum* (16.6 ± 4.13%) and *S. glaseri* (25.0 ± 7.25%). However, 100% mortality was observed only in case of *H. indica* within 120 HAI. *S. thermophilum* and *S. glaseri* could cause 70.9 ± 11.00 and 95.8 ± 4.13% of larval mortality within 120 HAI (Fig. 3.18 A-i) (Fig. 3.18 B-i) (Fig. 3.18 C-i).

At 25 IJs/larva, mortality was first observed again at 72 HAI for *H. indica* (50.0 ± 7.25%). However, first mortality was observed at 48 HAI for *S. thermophilum* (16.63 ± 4.13%) and *S. glaseri* (37.5 ± 7.25%). 100% mortality was observed for *H. indica* and *S. glaseri* within 120 HAI, *S. thermophilum* showed 91.6 ± 8.38% mortality

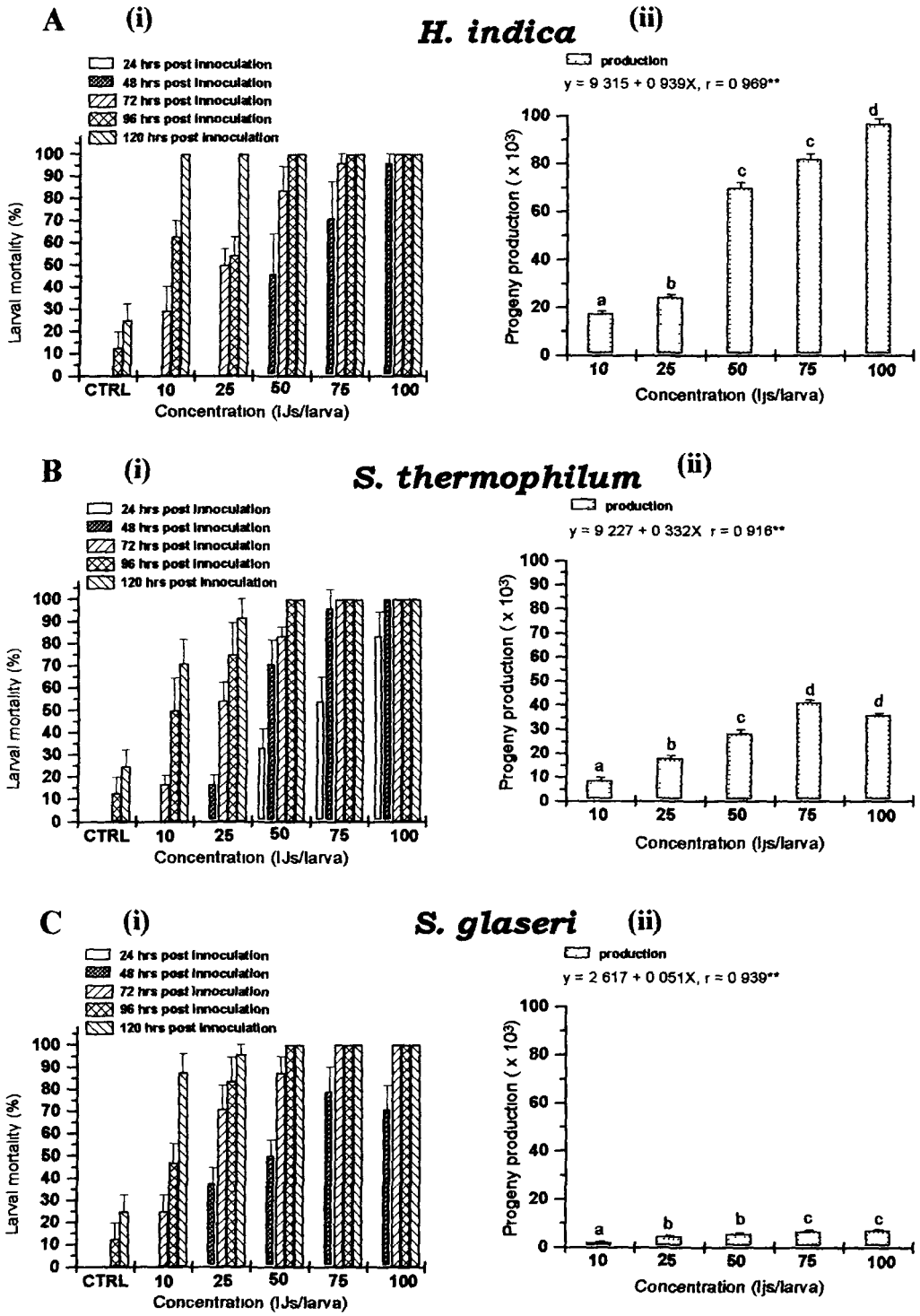


Figure 3.18 A-C: Bioefficacy of EPNs against ***L. orbonalis*** larvae.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
(i) Larval mortality (ii) Progeny production
***Significant at 0.01%* and **Significant at 0.05%*.*
Means shown by the same letter are not significantly different ($p < 0.05$).

Table 3.5: Correlations between the concentrations of EPNs and larval mortality time of *L. orbonalis*.

Concentrations (IJs/larva)	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
10	0.96**	0.95**	0.95**
25	0.95**	0.99**	0.96**
50	0.93**	0.93**	0.92*
75	0.84*	0.75	0.80*
100	0.72	0.70	0.83*

Time (hrs)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	0.98**	-
48	0.98**	0.95**	0.90**
72	0.94**	0.91*	0.86*
96	0.83*	0.85*	0.79
120	-	0.78	0.80

***significant at 0.01%* and **significant at 0.05%.*

Table 3.6: LC_{50} and LT_{50} values of EPNs against *L. orbonalis* larva.

Hours after inoculation	LC_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
24		**70.9**	-
48	**60.2**	**42.5**	**60.0**
72	**24.3**	**26.7**	**20.1**
96	**8.8**	**11.2**	**2.9**
120	-	**1.0**	-

Concentrations (IJs/larva)	LT_{50} (hrs)		
	H. indica	*S. thermophilum*	*S. glaseri*
10	**85.8**	**100.7**	**91.8**
25	**83.1**	**75.7**	**58.1**
50	**53.4**	**35.9**	**51.5**
75	**44.9**	**23.4**	**43.5**
100	**40.1**	**18.1**	**42.0**

within 120 HAI. At 50 IJs/larva, mortality was first observed at 48 HAI in case of *H. indica* (45.8 ± 18.13%) and *S. glaseri* (50.0 ± 7.25%), whereas it was observed at 24 HAI for *S. thermophilum* (33.3 ± 8.38%). 100% mortality was observed within 96 HAI for *H. indica* and *S. glaseri,* whereas it was at 72 HAI in *S. thermophilum.*

At 75 IJs/larva, *H. indica* and *S. glaseri* revealed first mortality of the host within 48 HAI and 100% mortality was noticed at 72 HAI. In contrast, first mortality was observed at 24 HAI in *S. thermophilum* which reached to 100% within 48 HAI. At 100 IJs/larva, no mortality of the host was observed in case of *H. indica* and *S. glaseri* within 24 HAI. First mortality of the host was observed within 48 HAI and 100% mortality was observed within 72 HAI for both the species. In case of *S. thermophilum,* first mortality was observed at 24 HAI and it reached to 100% within 48 HAI.

Mortality of insect larvae was also observed at control within 96 (12.5 ± 7.25%) and 120 (25.0 ± 7.25%) HAI. The calculated values of LC_{50} and LT_{50} are presented in Table 3.6.

Progeny production:

The production of IJs by larvae was strongly positively correlated with the concentrations for *H. indica (y = 9.315 + 0.939 x, r = 0.969)* (Fig. 3.18 A-ii), *S. thermophilum (y = 9.227 + 0.332 x, r = 0.916)* (Fig. 3.18 B-ii) and *S. glaseri (y = 2.617 + 0.051 x, r =*

0.939) (Fig. 3.18 C-ii). The production of IJs at different concentrations increased from 17.3 ± 1.09 x 10^3 IJs/larva at concentration of 10 IJs/larva to 96.7 ± 2.11 x 10^3 IJs/larva at 100 IJs/larva for *H. indica*; 8.7 ± 1.18 x 10^3 IJs/larva at concentration of 10 IJs/larva to 41.2 ± 0.97 x 10^3 IJs/larva at 75 IJs/larva for *S. thermophilum;* and 2.2 ± 0.21 x 10^3 IJs/larva at concentration of 10 IJs/larva to 7.2 ± 0.31 x 10^3 IJs/larva at concentration of 100 IJs/larva in case of *S. glaseri.*

Pupal mortality

The concentrations of IJs were found to be positively correlated with the time of pupal mortality of *L. orbonalis* (Table 3.7). At 25 IJs/pupa, the first mortality was observed at 48 HAI for *H. indica* (6.6 ± 6.6%) and *S. thermophilum* (13.4 ± 6.6%) (Fig. 3.19 A-i) (Fig. 3.19 B-i). The same reached to 60.0 ± 11.6 and 53.4 ± 13.4%, respectively within 120 HAI. No mortality was observed in case of *S. glaseri* (Fig. 3.19 C-i)

At 50 IJs/pupa, no mortality was observed again for *S. glaseri,* but in case of *H. indica* (20.0 ± 11.6%) and *S. thermophilum* (26.6 ± 13.4%), first mortality was observed at 48 HAI and the same reached to 66.6 ± 17.6 and 60.0 ± 11.6%, respectively within 120 HAI. At 75 IJs/pupa, mortality was first observed at 48 HAI in case of *H. indica* (73.4 ± 17.6%); 24 HAI in case of *S. thermophilum* (6.6 ± 6.6%), and 72 HAI for *S. glaseri* (6.6

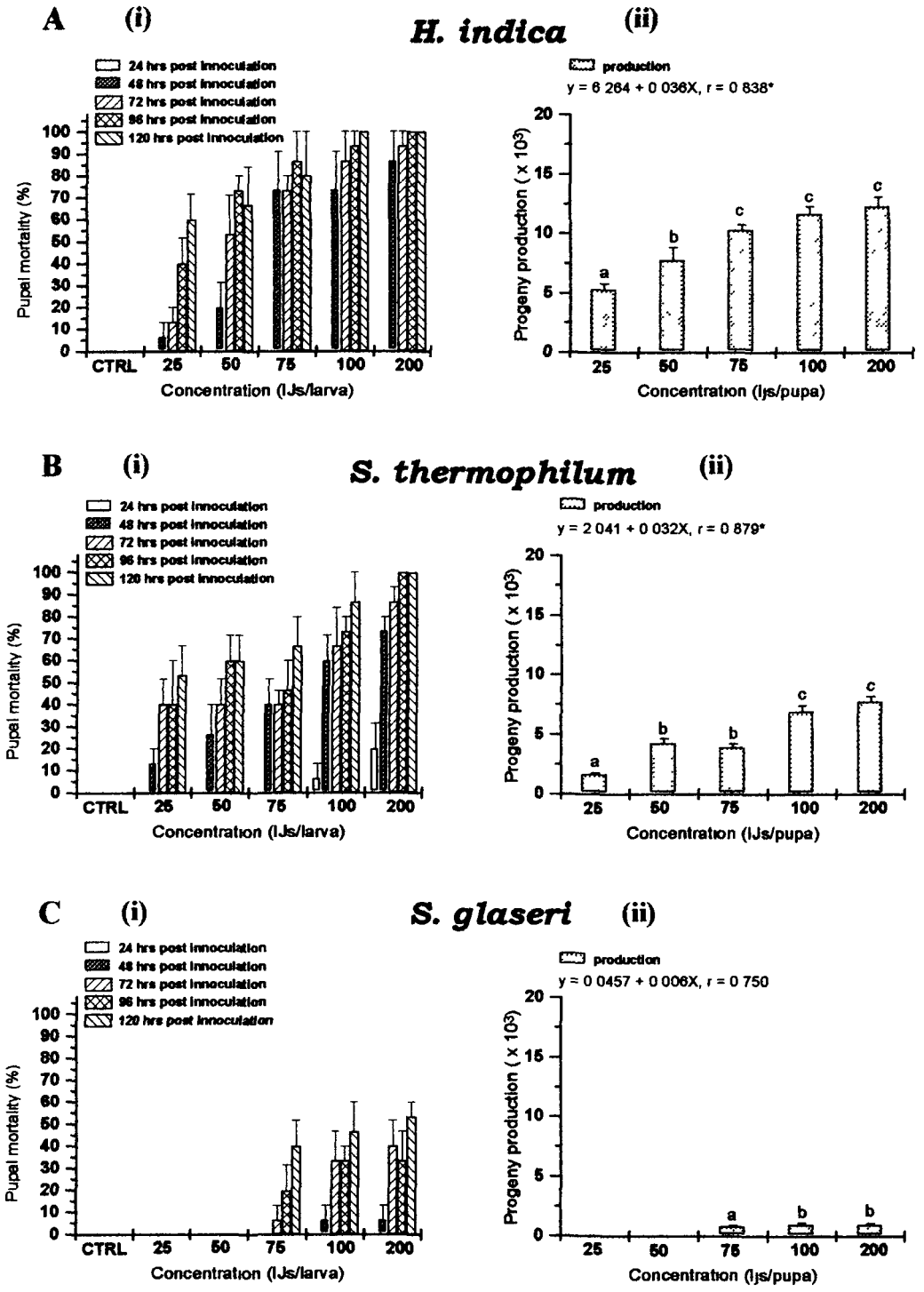


Figure 3.19 A-C: Bioefficacy of EPNs against ***L. orbonalis*** pupae
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
(i) Pupal mortality (ii) Progeny production
***Significant at 0.01%* and **Significant at 0.05%.*
Means shown by the same letter are not significantly different ($p < 0.05$).

Table 3.7: Correlations between the concentrations of EPNs and pupal mortality time of *L. orbonalis*.

Concentrations (IJs/larva)	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
25	0.96**	0.96**	-
50	0.93**	0.96**	-
75	0.77	0.91*	0.93*
100	0.85*	0.89*	0.95**
200	0.78	0.88*	0.93*

Time (hrs)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	0.96**	-
48	0.81*	0.93*	0.81*
72	0.80*	0.93**	0.89*
96	0.79*	0.93**	0.81*
120	0.84*	0.95**	0.81*

***significant at 0.01%* and **significant at 0.05%.*

Table 3.8: LC_{50} and LT_{50} values of EPNs against *L. orbonalis* pupa.

Hours after inoculation	LC_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	**270.4**	-
48	**81.8**	**111.0**	**413.0**
72	**55.0**	**72.2**	**205.2**
96	**31.0**	**50.6**	**224.2**
120	**20.9**	**28.9**	**159.8**

Concentrations (IJs/larva)	LT_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
25	**108.6**	**106.8**	
50	**83.6**	**92.7**	-
75	**54.1**	**92.0**	**126.7**
100	**48.0**	**59.7**	**116.4**
200	-	-	-

± 6.6%). Within 120 HAI, *H. indica* caused 80.0 ± 20.00% mortality, whereas *S. thermophilum* and *S. glaseri* caused 66.6 ± 13.4 and 40.0 ± 11.6% mortality, respectively.

At 100 IJs/pupa, in case of *H. indica* and *S. glaseri,* first mortality of the host was observed within 48 HAI and the same reached to 100% and 46.6 ± 13.4%, respectively within 120 HAI. In contrast, in *S. thermophilum* first mortality (6.6 ± 6.6%) was observed at 24 HAI which reached upto 86.6 ± 13.4% within 120 HAI. At 200 IJs/pupa, first mortality of the host was again observed within 48 HAI for *H. indica* and *S. glaseri.* 100% mortality was observed within 96 HAI in case of *H. indica.* Whereas, *S. glaseri* caused only 53.4 ± 6.6% mortality even within 120 HAI. In case of *S. thermophilum,* first mortality (20.0 ± 11.6%) was observed at 24 HAI which reached to 100% within 96 HAI.

No mortality was observed for control. Calculated values of LC_{50} and LT_{50} are shown in the Table 3.8.

Progeny production:

The production of IJs by *L. orbonalis* pupae was positively correlated with the concentrations for *H. indica (y = 6.264 + 0.036 x, r = 0.838)* (Fig. 3.19 A-ii), *S. thermophilum (y = 2.041 + 0.032 x, r = 0.879)* (Fig. 3.19 B-ii) and *S. glaseri (y = 0.046 + 0.006 x, r = 0.750)* (Fig. 3.19 C-ii). The production of IJs at different concentrations increased from 5.3 ± 0.49 x 10^3 IJs/pupa at

concentration of 25 IJs/pupa to 12.2 ± 0.78 x 10^3 IJs/pupa at 200 IJs/pupa for *H. indica.* For *S. thermophilum* it increased from 1.6 ± 0.09 x 10^3 IJs/pupa at concentration of 50 IJs/pupa to 7.7 ± 0.43 x 10^3 IJs/pupa at 200 IJs/pupa. In case of *S. glaseri* IJs production increased from 0.8 ± 0.05 x 10^3 IJs/pupa at concentration of 75 IJs/pupa to 0.9 ± 0.14 x 10^3 IJs/pupa at concentration of 100 IJs/pupa.

d) *Athalia lugens proxima*

Larval mortality

In this case, the observations were made till 72 HAI as the mortality rate of larva was considerably high at control (~ 50%) after 72 HAI. However, the concentrations of IJs and time duration showed a positive correlation with the mortality of *A. proxima* larvae (Table 3.9). At 10 IJs/larva, the first mortality was observed at 48 HAI for *H. indica* (29.1 ± 11.00%) and 83.3 ± 16.63% mortality was caused within 72 HAI (Fig. 3.20 A-i). In case of both *S. thermophilum* and *S. glaseri* first mortality was observed at 48 HAI. Both these species caused 41.6 ± 8.38% and 58.3 ± 8.38% mortality, respectively within 72 HAI (Fig. 3.20 B-i) (Fig. 3.20 C-i).

At 25 IJs/larva, mortality was first observed again at 48 HAI for all the species, but the mortality rate varied among the species. Within 72 HAI, *H. indica, S. thermophilum* and *S. glaseri*

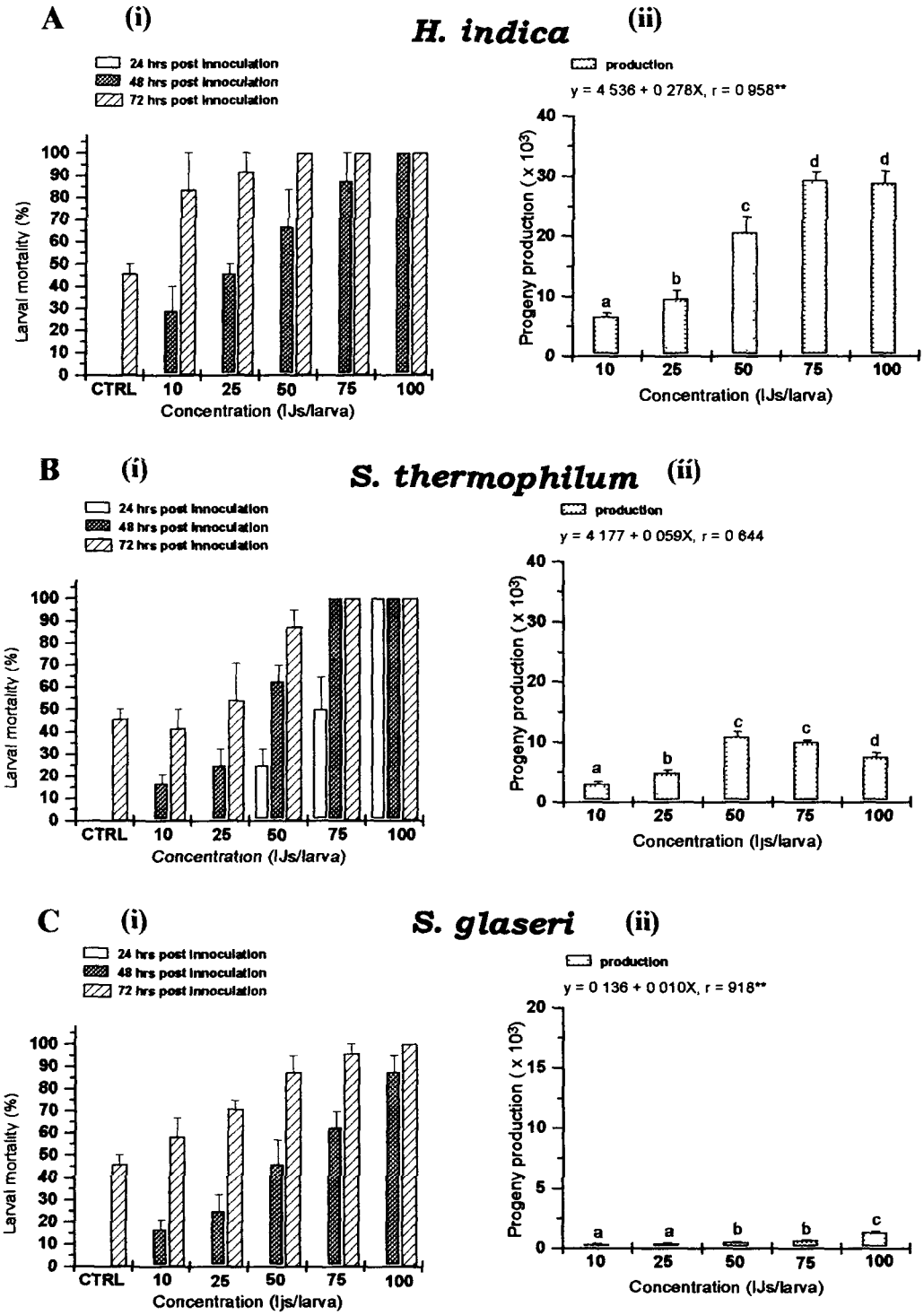


Figure 3.20 A-C: Bioefficacy of EPNs against ***A. proxima*** larvae.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
(i) Larval mortality (ii) Progeny production
***Significant at 0.01%* and **Significant at 0.05%.*
Means shown by the same letter are not significantly different ($p < 0.05$).

Table 3.9: Correlations between the concentrations of EPNs and larval mortality time of *A. proxima.*

Concentrations (IJs/larva)	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
10	0.98**	0.99**	0.97**
25	0.99**	0.99**	0.98**
50	0.98**	0.99**	0.99**
75	0.91*	0.86*	0.98**
100	0.86	-	0.91*

Time (hrs)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	0.96**	-
48	0.99**	0.96**	0.99**
72	0.85*	0.93**	0.96**

***significant at 0.01%* and **significant at 0.05%.*

Table 3.10: LC_{50} and LT_{50} values of EPNs against *A. proxima* larva.

Hours after inoculation	LC_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	**68.3**	-
48	**30.6**	**37.3**	**50.7**
72	-	**17.4**	**5.6**
96	-	-	-

Concentrations (IJs/larva)	LT_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
10	**64.0**	**81.3**	**70.5**
25	**51.4**	**73.7**	**65.7**
50	**45.6**	**38.2**	**52.4**
75	**39.0**	**24.0**	**46.1**
100	-	-	**39.0**

caused 91.63 ± 8.3%, 54.1 ± 16.63% and 70.8 ± 4.13% mortality of the host, respectively. At 50 IJs/larva, mortality was first observed at 48 HAI in case of *H. indica* (66.6 ± 16.63%) and *S. glaseri* (45.8 ± 11.00%), whereas it was observed at 24 HAI in case of *S. thermophilum* (25.0 ± 7.25%). At the same concentration, 100% mortality was observed within 72 HAI for *H. indica.*

At 75 IJs/larva, in case of *H. indica* and *S. glaseri,* first mortality of the host was observed within 48 HAI and 100% and 95.8 ± 4.13% mortality was observed within 72 HAI. In contrast, first mortality (50.0 ± 14.38%) was observed at 24 HAI which reached to 100% within 48 HAI in *S. thermophilum.* At 100 IJs/larva, again no mortality of the host was observed in case of *H. indica* and *S. glaseri* within 24 HAI. 100% mortality was observed at 48 HAI for *H. indica* but it was observed within 72 HAI in *S. glaseri.* In case of *S. thermophilum,* first mortality was observed at 24 HAI which reached to 100% within 48 HAI.

Mortality rate at control was 45.8 ± 4.13% within 72 HAI. Calculated values of LC_{50} and LT_{50} are presented in the Table 3.10.

Progeny production:

The production of IJs was positively correlated with the concentrations for *H. indica (y = 4.536 + 0.278 x, r = 0.958)* (Fig. 3.20 A-ii), *S. thermophilum (y = 4.177 + 0.059 x, r = 0.644)* (Fig. 3.20 B-ii) and *S. glaseri (y = 0.136 + 0.010 x, r = 0.918)* (Fig. 3.20 C-ii)). The production of IJs at different concentrations increased from 6.6 ± 0.55 x 10^3 IJs/larva at concentration of 10 IJs/larva to 29.3 ± 1.39 x 10^3 IJs/larva at 75 IJs/larva for *H. indica;* 2.9 ± 0.40 x 10^3 IJs/larva at concentration of 10 IJs/larva to 10.8 ± 0.79 x 10^3 IJs/larva at 50 IJs/larva and then declined to 7.6 ± 0.6 x 10^3 IJs/larva for *S. thermophilum;* and 0.4 ± 0.03 x 10^3 IJs/larva at concentration of 10 IJs/larva to 1.3 ± 0.12 x 10^3 IJs/larva at concentration of 100 IJs/larva in case of *S. glaseri.*

Pupal mortality

The concentrations of IJs were found to be positively correlated with the time of pupal mortality of *A. proxima* (Table 3.11). At 25 IJs/pupa, the first mortality of the pupa was observed at 96 HAI for *H. indica* (26.6 ± 6.6%) and *S. thermophilum* (13.4 ± 6.6%) which reached upto 33.4 ± 6.6 and 26.6 ± 6.6%, respectively within 120 HAI (Fig. 3.21 A-i) (Fig. 3.21 B-i). However, only 13.4 ± 6.6% mortality was observed for *S. glaseri* even within 120 HAI (Fig. 3.21 C-i). At 50 IJs/pupa, first mortality of the insect pupa was observed at 96 HAI for *H. indica* (33.4 ± 6.60%)

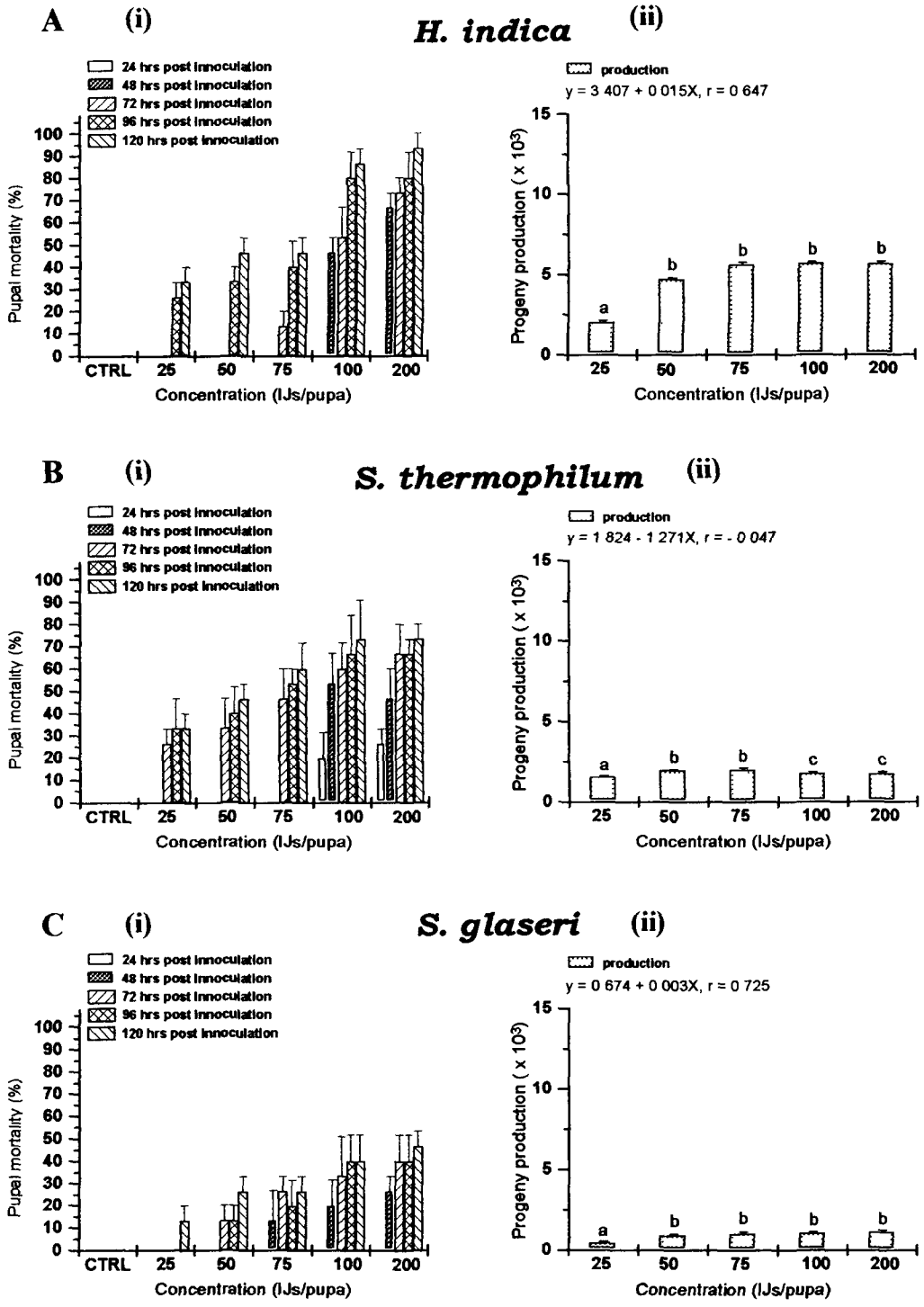


Figure 3.21A-C: Bioefficacy of EPNs against ***A. proxima*** pupae.
A. *H. indica* B. *S. thermophilum* C. *S. glaseri*
(i) Pupal mortality (ii) Progeny production
***Significant at 0.01%* and **Significant at 0.05%.*
Means shown by the same letter are not significantly different ($p < 0.05$).

Table 3.11: Correlations between the concentrations of *H. indica* and pupal mortality time of *L. orbonalis.*

Concentrations (IJs/larva)	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
25	0.88*	0.88*	0.70
50	0.89*	0.96**	0.94**
75	0.95**	0.89*	0.85*
100	0.95**	0.93*	0.93*
200	0.86*	0.96**	0.90*

Time (hrs)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	0.87*	-
48	0.90*	0.89*	0.90*
72	0.92*	0.51	0.87*
96	0.84*	0.69	0.84*
120	0.88*	0.81*	0.91*

***significant at 0.01%* and **significant at 0.05%.*

Table 3.12: LC_{50} and LT_{50} values of EPNs against *A. proxima* pupa.

Hours after inoculation	LC_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	**265.5**	-
48	**156.9**	**183.6**	**260.8**
72	**140.2**	**142.6**	**209.2**
96	**81.7**	**86.4**	**204.8**
120	**57.2**	**63.5**	**197.3**

Concentrations (IJs/larva)	LT_{50} (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
25	**127.4**	**135.8**	**130.1**
50	**117.4**	**103.6**	**148.7**
75	**115.3**	**86.9**	**164.7**
100	**68.2**	**73.7**	**120.9**
200	**56.1**	**70.6**	**112.3**

which reached up to 46.6 ± 6.60% within 120 HAI. In case of *S. thermophilum*, first mortality (6.6 ± 6.60%) was observed at 24 HAI and the same reached to 53.4 ± 6.60% within 120 HAI. *S. glaseri* caused first mortality (13.4 ± 6.60%) at 72 HAI which reached to 26.6 ± 6.60% within 120 HAI.

At 75 IJs/pupa, mortality was first observed at 48 HAI in case of *H. indica* (13.4 ± 6.6%); 24 HAI in case of *S. thermophilum* (13.4 ± 6.6%) and 72 HAI for *S. glaseri* (13.4 ± 13.4%). Within 120 HAI *H. indica* caused 46.6 ± 6.60% mortality, whereas *S. thermophilum* and *S. glaseri* caused 60.0 ± 11.60% and 26.6 ± 6.60% mortality, respectively. At 100 IJs/pupa, in case of *H. indica* and *S. glaseri*, first mortality (46.6 ± 6.60 and 20.0 ± 11.60%, respectively) of the host was observed within 48 HAI which reached to 86.6 ± 6.60% and 40.0 ± 11.60%, respectively within 120 HAI. In contrast, first mortality (20.0 ± 11.60%) was observed at 24 HAI and the same reached to 66.6 ± 6.60% within 120 HAI in case of *S. thermophilum*.

At 200 IJs/pupa, first mortality of the host was again observed within 48 HAI for *H. indica* and *S. glaseri*. 93.4 ± 6.60% mortality was observed within 96 HAI in case of *H. indica*, whereas *S. glaseri* caused only 46.6 ± 6.60% mortality even within 120 HAI. *S. thermophilum* showed first mortality (26.6 ± 6.60%) at 24 HAI which reached to 73.4 ± 6.60% within 96 HAI.

No mortality was observed for control. Calculated values of LC_{50} and LT_{50} are shown in the Table 3.12.

Progeny production:

The production of IJs by insect pupae was positively correlated with the concentrations for *H. indica (y = 3.407 + 0.015 x, r = 0.647)* (Fig. 3.21 A-ii), *S. thermophilum (y = 1.734 + 0.003 x, r = 0.646)* (Fig. 3.21 B-ii) and *S. glaseri (y = 0.674 + 0.003 x, r = 0.725)* (Fig. 3.21 C-ii). In case of *H. indica*, the production of IJs increased from 2.1 ± 0.09 x 10^3 IJs/pupa at concentration of 25 IJs/pupa to 5.7 ± 0.08 x 10^3 IJs/pupa at 100 IJs/pupa. In case of *S. thermophilum* the production was 1.8 ± 0.14 x 10^3 IJs/pupa at 25 IJs/pupa concentration and 2.2 ± 0.15 x 10^3 IJs/pupa at 75 IJs/pupa. *S. glaseri* revealed production of 0.5 ± 0.06 x 10^3 IJs/pupa at concentration of 25 IJs/pupa to 1.1 ± 0.10 x 10^3 IJs/pupa at concentration of 200 IJs/pupa.

Discussion

The nematodes in the genera *Heterorhabditis* and *Steinernema* are being used for the biological control of soil-dwelling insects (Grewal *et al.*, 2005). Interest in the promotion of EPN as biological agents for agricultural pests (Georgis and Manweiler, 1994) has provoked a number of studies on optimization of EPN for their bioefficacy (Zervos *et al.*, 1991). Various abiotic and biotic factors are known to influence the biocontrol efficacy of entomopathogenic nematodes (Womersley, 1990). Therefore a detailed knowledge of ecology of biocontrol agents is essential for its successful use. Koppenhofer and Kaya (1999) also emphasized the need to supplement the EPN species descriptions with data on ecological characterization and have developed protocols for such species. The studies made in this direction indicate that different nematode species have different environmental requirements (temperature, soil moisture, relative humidity, to name a few) depending upon place of their origin (Griffin, 1993; Koppenhofer and Kaya, 1999; Koppenhofer *et al.*, 2000). Therefore, it is essential to understand the optimum environmental conditions required for nematode species before testing their field efficacy in an area. Similarly, in order to formulate tests for the evaluation of nematodes against a particular host species, a better understanding of their pathogenesis is also essential (Glazer, 1992).

The aim of the present study was to consider the potential for use of three EPN species in the control of economically important insect pests in the area. Several important questions are addressed by this study including (1) what is the effect of temperature, soil moisture and relative humidity on infectivity and/or reproduction of infective juveniles (2) how foraging behaviour affects the attachment of IJs and infectivity at different soil depths to insect host (3) do population density of IJs have any impacts on reproduction of nematodes (4) what is the effect of chemical pesticides on the survival and pathogenicity of IJs (5) do these EPNs have any biocontrol potentials against economically important insect pests of the area.

Ecological characterization

Ecological characterization was undertaken for three locally isolated EPNs, namely *H. indica, S. thermophilum* and *S. glaseri.* The results suggest that the time taken for emergence of infective juveniles of all the species from the cadavers and their production was significantly influenced by temperature and relative humidity. The study also revealed that IJs of all the species did not establish at 10^{0}C. This finding gains support from Karunakar *et al.* (1999) who studied the temperature effects on *S. feltiae, S. glaseri* and *H. indica* and reported that no mortality of insect host was observed at 10^{0}C. In the present investigation it was observed that *H. indica* could establish and emerge at a temperature range of 15 to 30^{0}C, and the optimum temperature

range appeared to be 20 to 30°C which showed an earliest emergence of IJs (8 days post inoculation). At 35°C, though the IJs established, they failed to emerge during the entire 20-days period of observation. In a related study on the effect of different temperatures on infectivity and growth of *H. indica,* Sosamma (2003) reported that the fastest mortality of host occurs at 22 – 24°C. The optimum temperature range of *S. thermophilum* and *S. glaseri* establishment in the host was noticed to be between 25 – 35°C and 15 – 35°C, respectively. Further, the present study also recorded that unlike *H. indica* and *S. glaseri, S. thermophilum* did not establish at 15°C and its performance improves with the increasing temperature upto 35°C. Ganguly and Singh (2001) studied the optimal thermal requirement of *S. thermophilum* isolated from New Delhi and reported that following 120 hrs inoculation, the species does not cause any host mortality at 10°C and the same was only 25% at 20°C. They further observed that its mortality improves with the increasing temperature up to 35°C. It is worth mentioning here that while most species of *Steinernema* has not been found to develop and reproduce at temperature higher than 27°C (Kaya, 1990), a few like *S. abbasi, S. riobrave* and *S. puertoricencse* prefer high temperatures (Ganguly and Singh, 2001). It is therefore assumed that the thermal activity of *S. thermophilum* resembles with these three species as maximum number of IJs were produced at 30 and 35°C.

This study also recorded that unlike the temperature preference of *S. thermophilum, the S. glaseri* has a wider optimum temperature range (15-35^{0}C). Figueroa and Roman (1990) reported that *S. glaseri* performs well at temperatures between 20-31^{0}C to control *Diaprepes abbreviatus*. A slight difference in the temperature requirements of *S. glaseri* as observed in the present study may be attributed to its testing in different hosts or different origins of its isolates.

In the present study the first emergence of IJs and also their total production was noticed to be maximum at 25^{0}C for *H. indica* and *S. glaseri*, and at 30^{0}C for *S. thermophilum*. The 15^{0}C temperature delayed the emergence of *H. indica* and *S. glaseri* IJs and 20^{0}C in *S. thermophilum*. Grewal *et al.* (1994) also reported that exposure to low temperatures generally prolongs the time of emergence for both steinernematids and heterorhabditids. Comparisons of thermal thresholds to the geographical origins of rhabditid nematodes suggest a relationship between temperature niche breadth and locality and support the notion of temperature adaptation of rhabditid nematodes to their environment; e.g. tolerance to warmer temperatures by *S. riobravis*, *S. scapterisci*, *Steinernema* sp. (strain M87/45), *Heterorhabditis* sp. (strain Trinidad) and *H. indica* (strain 52) reflects their tropical origins (Grewal *et al.*, 1994; Mason and Hominick, 1995; Mason and Wright, 1997).

In the experiments on effects of relative humidity (R.H.) on reproduction of EPNs the first emergence of IJs and their total production within a limited time period was studied. The observations indicate that R.H. between 80 ± 5 and 100% show no significant difference on the emergence and total production of *H. indica.* However, at 70 ± 5% R.H. the emergence of IJs was found to be delayed. The delay in emergence observed in the present study may be due to desiccation of cadavers, which prevents nematodes to emerge out easily. In a related study, Subramanian and Sundarababu (2002) reported that at R.H. range of 57 - 69% and at temperatures 27 – 30.1°C for 12 hrs, only 2% of *H. indica* survived. In case of *S. thermophilum* and *S. glaseri* though there was no difference in the first emergence of IJs at different R.H., the total production increased with increasing relative humidity. Wharton (1995) emphasized that low temperature and relative humidity combinations may intensify the effects of one or both factors on nematode survival. Low humidity could slow or prevent emergence and increase the chance of the nematodes becoming trapped in the desiccating cadaver. It is worth mentioning here that the optimum R.H. range for all the species (80–100%) observed in the study corresponds well to the prevailing R.H. of the area where the three species were isolated.

One of the important steps in the utilization of EPNs for biocontrol programme is the storage of EPN infective juveniles in a given

population density at optimum temperature, so that maximum IJs can survive for long periods before they are utilized for field application. In the present study, the best combination for storage was observed to be 100 IJs/ml and 10^{0}C temperature for *H. indica* and *S. glaseri*, and 25^{0}C and 100 IJs/ml for *S. thermophilum*. At these combinations the storage durability was noticed to be best with a time less than 60 days. After 60 days, though the nematodes survived but their infectivity was significantly lowered in all the species. Poinar (1979) reported that the favorable temperature range for storage of *Steinernema* sp. to be 5 – 9^{0}C. Bedding (1981) reported that 12^{0}C was the best for storage of *Heterorhabditis* sp. in culture flasks. Karunakar *et al.* (2001) reported that the best combination for storage was 7.5^{0}C, 250 IJs/ml within 120 days for *S. feltiae;* 7.5^{0}C, 250 IJs/ml within 90 days for *S. glaseri;* and 10^{0}C, 250 IJs/ml within 90 days for *H. indica.* A slight difference observed may be due to different geographical origins of EPNs isolates. It may also be assumed that being a warm adapted nematode, *S. thermophilum* could not survive long periods at low temperatures and its infectivity is affected at low temperatures. It therefore emerges from this study that though heterorhabditids and steinernematids can be stored for more than 2 months, but for use as biological control agents they should not be stored more than 2 months as their infectivity lowers after this time period.

In seeking new hosts, different entomopathogenic nematode species employ foraging strategies ranging from sit-and-wait foragers (ambushers) that search in one place through time to widely active foragers (cruisers) that search by moving through their environment (Campbell and Gaugler, 1993). *S. carpocapsae* is an ambusher that nictates (Kondo and Ishibashi, 1986), stays near the soil surface and does not disperse far (Moyle and Kaya, 1981), is unresponsive to host cues (Lewis *et al.*, 1992), and is adapted to infecting mobile hosts on the soil surface (Campbell and Gaugler, 1993). On the other hand, cruisers like *S. glaseri* actively move in the soil (Schroeder and Beavers, 1987), respond strongly to host cues (Lewis *et al.*, 1992), and are adapted to infecting sedentary hosts (Campbell and Gaugler, 1993). It therefore becomes necessary to study the attachment rate of EPNs to their host. In this study, the attachment rate of IJs to the host varied and was found to be dependent on time duration. Among the three species, attachment of IJs of *H. indica* to the mobile host was high as compared to IJs of *S. thermophilum* and *S. glaseri* at all observation time. However, significant differences in attachment of IJs to host were observed between *S. thermophilum* and *S. glaseri* at longer time periods.

The infection profile of EPN at different soil depths also varied among the species. For all the EPNs the establishment rate of IJs to the host declined as the soil depth increased. *H. indica* showed highest infection at 2 cms soil depth but unlike the two Steinernema spp. failed

to establish at 10 cms soil depth. *S. glaseri* showed highest infection at both 5 and 10 cms soil depths. No significant difference on infection was observed between *S. glaseri* and *S. thermophilum* at all the soil depths. The present investigations thus suggest that *H. indica* probably uses an intermediate foraging strategy while, *S. thermophilum* and *S. glaseri* make use of a cruising type foraging strategy.

The survival, motility and the ability of EPN infective juveniles to penetrate into a proximal host to a larger extent also depend upon soil moisture. An attempt was therefore made to investigate the impacts of soil moisture on establishment of three EPN species. The study revealed that the infective juveniles of *S. thermophilum* establishes at lower soil moisture level (4%) as compared to *H. indica* and *S. glaseri*. At 5% moisture level IJs establishment was observed for all the species though their mortality varied considerably. The number of IJs established per host increased with increase in soil moistures, reaching their peak at 16 – 18% soil moisture level and declined from 20% onwards. It is therefore evident from the study that soil moisture greatly influences the number of IJs established in the host and also the mortality of insect larvae at different soil moisture levels. Koppenhofer *et al.* (1995) in their study on effect of different soil moisture levels on pathogenicity of *S. carpocapsae* and *S. glaseri* reported that a considerable establishment of IJs of *S. carpocapsae* occurs at 4 to 5% moisture levels. They further noted that *S. glaseri* does not establishes at 3% moisture level and a considerable

establishment was observed at 4% and further the highest rate of IJs establish was observed at 19% moisture level. In another related study, Koppenhofer *et al.* (2000) also reported that IJs of *S. monticolum* did not infect wax moth larvae at 2 and 3% moisture levels. In this case the establishment starts at 3.5% and reaches its peak at 6% with very low establishment at 19%. It may be hypothesized that the size of IJs could also be an important factor in determining their establishment at different soil moisture levels. In the current study, *S. glaseri* being the largest EPNs studied (IJs length > 800 µm), established successfully at higher moisture levels. In contrast, the smaller sized IJs like *H. indica* and *S. thermophilum* established more at lower soil moisture levels but their establishment declined at higher soil moisture levels. This may be due to the fact that larger IJs need high soil moisture level (thicker water film) to move freely and infect the host successfully than the smaller IJs. The findings of present study further gain support from the views of Womersely (1990) who stated that the infective stages of steinernematid and heterorhabditid nematodes are exclusively associated with the microenvironment provided by interstitial spaces of the soil, the water dynamics of which vary depending on moisture availability. It is obvious from the study that differences in establishment of IJs and mortality of insect larva by different EPNs may be due to the climatic origins or the soil habitats of these strains. These

factors therefore need a thorough investigation to optimize the infectivity potentials of EPNs studied.

The development of parasites in a host may be adversely affected by high infection density. Changes in sex ratio, reduction in body size, progeny production and survival of entomogenous nematodes have been attributed to crowding of the parasites in their host. (Hominick and Tingley, 1984; Tingley and Anderson, 1986). This study observed that concentrations of IJs had a significant effect on the numbers of IJs established per host, which in turn affect their reproduction potential for all the species. The percentage of the initial inoculum of IJs established in host decreased linearly with the increase in concentrations for all the species. This observation agreed with Selvan *et al.* (1993) who reported that the proportion of *S. carpocapsae* and *H. bacteriophora* infecting *G. mellonella* larvae declines with increasing dose. Danilov (1987), using longer exposure periods, also found that *S. carpocapsae* percentage infection declines with increasing dose. In contrast, Fan and Hominick (1991) found that *Heterorhabditis* sp. percentage infection was relatively constant over a range of doses. However, they used a relatively low dose range of IJs (10 – 300) and a series of host exposures. Within this dose range a similar proportion of host infection was also found. With respect to first day of emergence of IJs from cadavers, the study revealed that in general, earlier emergences of IJs occur at higher concentration densities.

It was also observed that IJs production for the three species increased with increasing initial density up to 200 IJs/larva approximately and then declined. The highest number of IJs production noted was maximum for *H. indica* (183 ± 6.21 x 10^3 IJs) followed by *S. thermophilum* (101.8 ± 3.02 x 10^3 IJs), and the same was considerably very low (15.3 ± 1.33 x 10^3 IJs) in *S. glaseri*. The lower progeny production at high infection densities has also been observed for other steinernematids and heterorhabditids by Sandner and Stanuzek (1971) and Molyneux *et al.* (1983). Selvan *et al.* (1993) while studying *S. carpocapsae* and *H. bacteriophora* found an optimum progeny production at 103 and 128 nematodes established/wax moth larva respectively, but numbers of cadavers failed to produce IJs above densities of 150 IJs/host in case of *S. carpocapsae*. Zervos *et al.* (1991) observed that the number of progeny produced by *S. glaseri* in wax moth larvae was affected by inoculum level and temperature but did not provide the actual number of IJs entering the insect host and they limited their study to relatively low inocula.

Chemical insecticides and EPNs offer different but potentially compatible approaches to suppress insect populations (Nishimatsu and Jackson, 1998). The EPNs may seek out the host in inaccessible areas where pesticides may not act. It is therefore advocated that efforts should be made to utilize the EPNs with existing IPM practices. Though, compatibility of nematodes with chemicals have been conducted as

laboratory bioassays with direct exposure of nematodes to insecticides (Hara and Kaya, 1982, 1983; Rovesti *et al.*, 1988; Rovesti and Deseo, 1990; Zhang *et al.*, 1994; Gordon *et al.*, 1996), it is still necessary to test the chemicals effects on the nematodes, because formulations of different insecticides may vary in toxicity to nematodes due to the use of different surfactants. Further, nematodes species may also differ in sensitivity to different formulations of the same insecticide (Rovesti and Deseo, 1990; Krishnayya and Grewal, 2002).

In the present study, no significant differences were observed on survival of IJs of *H. indica* when exposed to mancozeb and nimbecidine at both lower and higher concentrations after 72 hrs, whereas other insecticides, particularly quinolphos, affected the survival and infectivity of *H. indica*. Hussaini *et al.* (2001) also reported that pesticides such as, endosulfan, carbofuran and malathion have deleterious effects on *H. indica*. A reduction in the movement of EPN IJs, in general, by endosulfan has also been reported by Rovesti and Deseo (1990). In case of *S. thermophilum* the study revealed that barring quinolphos and fenvalerate which showed deleterious effects, the other pesticides did not show significant effects on survival and pathogenicity of its IJs. The IJs of *S. glaseri* showed a high rate of survival when exposed to nimbecidine, endosulfan, quinolphos, fenvalerate and mancozeb. However, carbaryl and carbofuran had serious effects on survival and infectivity of IJs of *S. glaseri*. It is relevant to mention here that Hussaini

et al. (2001) have also reported that carbofuran impaired the activity of steinernematids. Hussaini *et al.* (2001) have also cited that *S. glaseri* to be compatible with quinolphos. The chemical groups most toxic to steinernematids and heterorhabditids are thought to be organophosphates and carbamates (Rovesti and Deseo, 1990; Gordon *et al.*, 1996). However, it has been suggested that in some cases simultaneous use of nematodes and insecticides may improve insect pest control (Ishibashi, 1992). Though, it was reported in this study that nimbecidine and mancozeb were compatible for all the nematode species, Krishnayya and Grewal (2002) reported that neem formulation, nimbecidine, cause 14 – 17% mortality on IJs of *S. feltiae* after exposure of 24 hrs. Water extract of neem seed kernel was also reported to be toxic to *S. carpocapsae* (Rovesti and Deseo, 1989). The differences in the toxicity of nimbecidine/or neem extract to these nematodes may be attributed to different species used in the assay.

It can be concluded that, though IJs of the nematodes were not compatible with few pesticides, most of others showed positive results and hence could be considered compatible with EPNs. The study therefore suggests that if it is necessary to use these pesticides with EPNs their doses be kept as low as possible. The entomopathogenic nematodes tested in this study, therefore, may be viable candidates for integrated pest management.

Bio-efficacy of EPNs against Pests

The efficacy of various EPN species or strains for controlling a particular insect pest may differ significantly (Forschler and Nordin, 1988; Kondo and Ishibashi, 1988). The need to evaluate insecticidal activity in the laboratory has resulted in the development of a variety of assays that measure nematode infectivity by host mortality (Caroli *et al.*, 1996). One such assay which has been commonly applied in many studies includes dose-response test, i.e., to determine the percent mortality of insect pest following its exposure to different doses of IJs for different time periods (Fan and Hominick, 1991). Once the rate of pest mortality is established, one can also go for reproduction capability tests by accessing the total number of IJs of the test EPNs emerging from the dead pest in a limited time period. The assessment of reproduction capability of EPN becomes important as its gives an idea about total production of IJs that can emerge from pest and in turn can recycle in the environment to infect other surrounding pests. The objective of this study was to explore the biocontrol potentials of EPN species, *H. indica, S. thermophilum* and *S. glaseri* against four economically important pests of the area with respect to above parameters. The four insect pests comprised of *Haplosonyx chalybaeus* (Colocasia corm borer), *Pieris brassicae* (Cabbage butterfly), *Leucinodes orbonalis* (Brinjal fruit and shoot borer) and *Athalia lugens proxima* (Mustard sawfly).

This study reports the first data on susceptibility of larva of colocasia corm borer, *H. chalybaeus* to entomopathogenic nematodes. Mortality of *H. chalybaeus* varied when exposed to different nematode species at different concentrations of IJs. Similarly, the number of IJs produced/larva were highly influenced by concentrations of IJs. The study revealed that *S. glaseri* was the most potent nematode among the three, as it caused 100% mortality at 75 IJs/larva within 96 hrs (LC_{50} = 46.2 ; LT_{50} = 60.0). *H. indica* caused 100% mortality at 100 IJs/larva within 120 hrs (LC_{50} = 48.7; LT_{50} = 66.3), whereas 100% mortality was noticed at 200 IJs/larva within 72 hrs in case of *S. thermophilum* (LC_{50} = 74.4; LT_{50} = 18.8). On the basis of LC_{50} values, *S. glaseri* also emerged to be the most promising EPN as compared to the others.

Following exposure of *H. chalybaeus* larvae to different concentrations of IJs, all the nematode species were found to propagate inside the infected host. It appears that the number of IJs produced by larvae depends on the nematode size and also species, besides other factors. *H. indica* which is the smallest among the three EPN species produced more IJs/larva. In contrast, *S. glaseri* which was the largest produced the least IJs/larva. Bhatnagar *et al.* (2004) reported similar results while studying EPN against white grub, *Maladera insanabilis*. They mentioned that both the size of IJs and the relative virulence of particular nematode species/strains to a specific insect host determine the number of IJs produced per larva.

In the present study, the larvae of cabbage butterfly, *P. brassicae* were also found to be very susceptible to EPNs. Though, all the nematode species tested caused 100% mortality, their infectivity levels varied. Among the three species *S. thermophilum* appeared to be the most potent one as it caused larval mortality at 24 HAI at 50 IJs/larva. However, at 48 HAI in addition to *S. thermophilum, H. indica* also revealed 100% mortality at 100 IJs/larva. In case of *S. glaseri* no mortality was observed at 24 HAI. The species showed a 100% larval mortality at 120 HAI at 50 IJs/larva. The study thus concludes that both on the basis of mortality and LC_{50} value (30.2 IJs/larva at 48 HAI) *S. thermophilum* emerges as the most potent species.

Of all the EPNs, the progeny production by larvae of *P. brassicae* was noted to be highest in case of *H. indica.* The same was noticed to be considerably low in case of *S. thermophilum* and *S. glaseri.* The production increased along the concentrations till the highest concentration for both *H. indica* and *S. thermophilum* but declined from 50 IJs/larva onwards in case of *S. glaseri.* These observations are in agreement with the findings of Mahar *et al.* (2005), who studied the production and infectivity of *S. carpocapsae, S. feltiae, H. indica* and *H. bacteriophora* against *P. brassicae* and reported that the progeny production was highest in case of *H. indica* as compared to the other species.

With respect to efficacy of EPNs against *L. orbonalis* larvae 100% mortality of host was observed at 10 IJs/larva concentration within 120 HAI only in case of *H. indica*. Whereas, *S. thermophilum* and *S. glaseri* could cause a 100% mortality within 96 HAI at 50 IJs/larva. In terms of time duration, only *S. thermophilum* revealed an early mortality of larvae (24 HAI) at 50 IJs/larva. Hussaini *et al.* (2002) while reporting the susceptibility of *L. orbonalis* larvae to entomopathogenic nematodes reported that *H. indica* causes 100% mortality within 72 hrs at 25 IJs/larva. However, comparisons of LC_{50} values of EPNs at 50 IJs/larva within 96 HAI reveal *S. glaseri* to be the potent EPN.

The pupae of *L. orbonalis* were less susceptible to EPNs as compared to larvae. The mortality of pupae was moderate at 25 to 75 IJs/pupa concentration in case of *H. indica* and *S. thermophilum*. No pupal mortality was observed for *S. glaseri* at 25 to 50 IJs/pupa. 100% mortality was observed at 200 IJs/pupa within 96 HAI in case of *H. indica* and *S. thermophilum*. However, at 200 IJs/pupa S. *glaseri* could cause approximately 50% mortality within 120 HAI. A possible reason for low mortality of pupae by EPNs may be due to low penetration of IJs into their body. The pupal stages have their own puparium which create a barrier for the IJs to enter (Toledo *et al.*, 2001).

From a pest management standpoint, the main goal is to kill a large number of the target pests to bring it below the economic threshold level; however, if nematode reproduction can occur

successfully in the target insect, then longer term management might be achievable. The present study observed that the EPNs reproduction performance is better in larvae as compared to pupae. In general, a high progeny production was recorded in case of *H. indica* which was followed by *S. thermophilum*. *S. glaseri* did not reveal a better progeny production.

In case of mortality assay of *A. proxima* the observations were made till 72 HAI as the mortality rate of larva was considerably high at control (~ 50%). The study showed that at 10 and 25 IJs/larva the mortality caused by *Steinernema* spp. was almost comparable to mortality observed in control. However, at 75 IJs/larva within 72 HAI the mortality was observed to be 100% in case of *H. indica* and *S. thermophilum* and 96% for *S. glaseri*. On the basis of comparisons of LC_{50} values at 48 HAI, *H. indica* emerged to be the most effective species. Narayanan and Gopalakrishnan (2003) in their study on susceptibility of A. *proxima* by *S. feltiae* have reported a high mortality rate of the larvae.

In case of *A. proxima* pupae, *H. indica* and *S. thermophilum* showed almost similar efficacy as compared to *S. glaseri* which revealed a considerably low profile of pupal mortality. The progeny production by *A. proxima* pupae was noted to be quite low as compared to its larvae. Differences in the susceptibility among insect life-cycle stages have also been observed in the family Pyralidae, with the pupae being less

susceptible than the larvae (Shannag and Capinera, 1995; Shannag *et al.*, 1994).

In summarizing the findings, it can be stated that in terms of biocontrol agents *H. indica* appears to be the most promising EPN, followed by *S. thermophilum* and *S. glaseri* against the four economically important pests of the area, *Haplosonyx chalybaeus, Pieris brassicae, Leucinodes orbonalis* and *Athalia lugens proxima.* Further, these EPNs showed good compatibility with most of the tested pesticides. Hence they could also be incorporated into the integrated pest management strategies to control insect pests. It is suggested that in order to estimate the practical value of applying these nematodes in the control of insect pests field studies should be continued.

SUMMARY

Entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinenernema* spp.) are promising biological control agents for a variety of soil-dwelling insect pests. The present work deals with a study on ascertaining the faunal composition, distribution, ecological characterization and bioefficacy of entomopathogenic nematodes in the Ri-bhoi District of Meghalaya. The objectives of study were:

1. To ascertain the faunal composition and distribution of EPNs in Ri-bhoi District of Meghalaya.
2. To study the seasonal prevalence of EPNs.
3. To characterize the EPN species with respect to ecological parameters.
4. To test the bioefficacy of locally isolated EPN species against major insect pest in the area.

To study the occurrence and distribution of EPNs in the area, soil samples from different habitats (Dry land, Wet land, Jhum land and Forest land) were collected and baited by *Galleria* traps. Entomopathogenic nematodes were recorded from 89 samples (5.37%) out of 1656 samples collected from various habitats. Out of 89 positive samples, the frequency of occurrence of *Steinernema* spp. was recorded to be more (73.03%) than *Heterorhabditis* sp. (26.97%). All the EPN positive

samples were from the forest soils. *Steinernema* spp. were mostly isolated from sandy loam soils while *Heterorhabditis* sp., was isolated from red loamy soil.

Based on their morphometric measurements, and light/scanning electron microscopic studies the isolated nematodes were identified as, *Heterorhabditis indica, Steinernema thermophilum* and *Steinernema glaseri.* It is for the first time that their occurrence is reported herein from north-east region of India, Meghalaya in particular. A brief description is provided for all the species recorded.

For the study of seasonal population fluctuations of EPNs, two nematode positive sites, one for *Heterorhabditis* sp. and another for *Steinernema* sp. were selected in the study area. The seasonal prevalence was studied using indirect method and the presence of EPNs was evaluated using *Galleria* traps. *Heterorhabditis* sp. was detected in the soil samples throughout the study period, causing moderate to high mortality of the host insect. The prevalence of *Heterorhabditis* and *Steinernema* species was found to be positively correlated with soil temperature, soil moisture and rainfall. The prevalence of *Heterorhabditis* was recorded to be considerably high during March to November. The prevalence of *Steinernema* sp. started increasing form March onwards and reached a peak in May; thereafter it maintained almost a uniform trend till November. The prevalence declined abruptly to a very low level in the month of December.

With respect to ecological characterization of EPN species, effects of following parameters were taken into consideration: 1). temperatures on infectivity/production of IJs 2.) relative humidity on production of IJs 3). storage temperatures, population densities and duration on survival and pathogenicity of IJs 4). Time period and soil depth on attachment of IJs to a mobile host 5.) soil moisture levels on establishment of IJs in the host 6). IJs population densities on their infectivity/production 7). different pesticides on survival/pathogenicity of IJs.

Effect of temperatures on infectivity and reproduction of EPNs was determined by numbers of IJs established per *G. mellonella* and the numbers of IJs produced per insect host at different temperatures (10 – 35^{0}C). The optimum temperature (high establishment and production of IJs) of EPNs were recorded to be, *H. indica* from 20–30^{0}C, *S. thermophilum* from 25–35^{0}C and *S. glaseri* from 15-35^{0}C.

Effect of relative humidities on reproduction of IJs was determined by the first day of emergence of IJs and the number of IJs produced by the insect host exposed to 55 – 100% R.H. The optimum R.H range for all three species was found to be 80 – 100%.

To study the effect of storage temperature, population densities and duration, IJs of EPNs were exposed to four different temperatures (5, 10, 25 and 30^{0}C) and three population densities (100, 500 and 1000 IJs/ml) for a period of 120 days and their survival and pathogenicity were determined at different time intervals. Study revealed that 10^{0}C, 100

IJs/ml but not more than 60 days was the optimum condition for *H. indica* and *S. glaseri,* whereas 25°C, 100 IJs/ml but not more than 60 days was the best condition for *S. thermophilum.*

Foraging behavior of isolated EPNs was studied by two methods 1). attachment of IJs to a mobile host 2). capability of IJs to infect host at different soil depths. The attachment *H. indica* IJs to a mobile host was higher at all observation time (1, 5 and 10 minutes) as compared to *S. thermophilum* and *S. glaseri.* However, at different soil depths *S. glaseri* appeared as the most effective species as it showed the highest establishment of IJs in the host at the deepest soil depth of 10 cms.

To study the effect of soil moisture on infectivity of IJs, establishment of IJs in the insect host was observed at different soil moisture levels. It emerged that IJs of *S. thermophilum* could establish at 4% soil moisture level. At 5% moisture level IJs establishment was observed for all EPNs. Numbers of IJs established/host increased along the soil moistures reaching their peak at 16 – 18% soil moisture level and declined from 20% onwards, except for *S. glaseri* where the establishment rate increased till the highest soil moisture level studied.

The effect of IJs population densities on their infectivity and production were studied by exposing *G. mellonella* larvae to different concentrations of IJs. The infected dead larvae were observed for the establishment, first day of emergence and production of IJs of EPNs. It appeared that the number of IJs established per insect host significantly

increases at higher concentrations for all the EPNs. The first day of IJs emergence from the host cadavers was also affected by the population densities. Earlier emergences were observed at higher densities but no IJs were produced at establishment of IJs higher than 551.5 ± 41.92, 428.4 ± 74.73 and 321.4 ± 22.96 IJs per host for *H. indica, S. thermophilum* and *S. glaseri,* respectively. The highest numbers of IJs were produced at a concentration of 200 IJs/larva for both *H. indica* and *S. thermophilum.*

In order to ascertain the effect of different pesticides on survival and pathogenicity, IJs of EPNs were exposed to two (lower and higher) recommended field dose of carbaryl, nimbecidine, endosulfan, quinolphos, fenvalerate, mancozeb and carbofuran for a period of 72 hrs. It was observed that IJs of *H. indica* was found compatible with mancozeb and nimbecidine at both lower and higher concentrations. IJs of *S. thermophilum* were found compatible with carbaryl and nimbecidine at lower and higher concentrations, and to mancozeb at lower concentration, whereas IJs of *S. glaseri* were found compatible with nimbecidine, endosulfan, quinolphos, fenvalerate and mancozeb.

Bioefficacy of the isolated EPNs were tested against four major insect pests of the study area, namely larvae of colocassia corm borer, *Haplosonyx chalybaeus,* larvae of cabbage butterfly, *Pieris brassicae,* larvae and pupae of brinjal fruit and shoot borer, *Leucinodes orbonalis* and larvae and pupae of mustard saw fly, *Athalia lugens proxima.* Bioefficacy

was determined by percent insect larval/pupal mortality, and the total production of IJs per host to ensure their recycling potential.

The bio-efficacy of nematode species against *H. chalybaeus* larvae was studied by petridish assay. The larvae were exposed to 25, 50, 75, 100 and 200 concentrations of IJs for 120 hrs and host mortality was monitored at every 24 hrs intervals. 100% of larval mortality was observed for all EPNs at a concentration of 200 IJs/larva within 48 hours after inoculation (HAI) in case of *S. glaseri* and 72 HAI in case of both *H. indica* and *S. thermophilum*. Progeny production increased along with the IJs concentrations in EPN species. The maximum number of IJs produced was observed in case of *H. indica* (168.9 ± 2.67 x 10^3 IJs/larva at 200 IJs/larva) whereas the least infective juveniles yield was observed in case of *S. glaseri* (18.9 ± 0.57 x 10^3 IJs/larva at 100 IJs/larva).

Bioefficacy against larvae of *P. brassicae* was also studied using petridish assay with 10, 25, 50, 75 and 100 IJs concentration for a period of 120 hrs. The insect mortality was monitored at every 24 hrs intervals. Positive correlation was observed between the concentrations and the time of insect larval mortality. 100% of insect larval mortality was observed for all EPNs at a concentration of 200 IJs/larva within 48 HAI in case of *S. glaseri* and 72 HAI in case of both *H. indica* and *S. thermophilum*. The highest number of infective juveniles produced by *P. brassicae* was observed in case of *H. indica* (33.8 ± 2.46 x 10^3 IJs/larva at 100 IJs/larva).

In order to evaluate the bio-efficacy of nematode species against larvae and pupae of *L. orbonalis,* two assays were carried out *i.e.,* petridish assay for larvae and soil column assay for pupae. The larvae and pupae were also found to be susceptible to EPN species when exposed to different concentrations (10, 25, 50, 75 and 100 IJs/larva and 25, 50, 75, 100 and 200 IJs/pupa). In case of larvae, at the lowest concentration studied (10 IJs/larva), 100% mortality was observed only in case of *H. indica* within 120 HAI. At higher concentrations (100 IJs/larva) 100% mortality was observed for all the species within 48 HAI. The production of IJs by *L. orbonalis* showed the same trend as with other insect pests, where the maximum production of IJs was observed in case of *H. indica* (96.7 ± 2.11 x 10^3 IJs/larva at 100 IJs/larva).

Pupae of *L. orbonalis* were less susceptible to EPNs. At the highest concentration studied (200 IJs/larva), 100% mortality was observed within 96 HAI in case of *H. indica* (LC_{50} = 31.0), whereas *S. glaseri* caused only 53.4 ± 6.6% mortality even within 120 HAI (LC_{50} = 159.8). In case of *S. thermophilum,* an early mortality (20.00 ± 11.6%) was observed at 24 HAI which reached to 100% within 96 HAI (LC_{50} = 50.6). The pupa produced comparably less IJs than larva. Among the three species the production was observed highest in *H. indica,* yielding 12.2 ± 0.78 x 10^3 IJs/pupa at 200 IJs/pupa.

Bioefficacy against larvae and pupae of *A. proxima* were also studied by two assays (petridish assay for larvae and soil column assay for pupae)

at 10, 25, 50, 75 and 100 IJs/larva and 25, 50, 75, 100 and 200 IJs/pupa concentrations. In case of larvae, at the highest concentration (100 IJs/larva), 100% mortality was observed at 48 HAI for *H. indica* (LC_{50} = 30.6), whereas it was observed within 72 HAI (LC_{50} = 5.6) in *S. glaseri.* In case of *S. thermophilum,* mortality was first observed at 24 HAI which reached to 100% within 48 HAI (LC_{50} = 37.3). Of all the insect pest studied, the production of EPN IJs was comparably less in *A. proxima* larvae. The highest IJs produced were found to be only 29.3 ± 1.39 x 10^3 IJs/larva at 75 IJs/larva in case of *H. indica.*

The pupae were found less susceptible to EPNs as compared to larvae. No species could cause 100% pupal mortality. However, at the highest concentration of IJs (200 IJs/pupa), 93.4 ± 6.60% mortality was observed within 96 HAI in case of *H. indica* (LC_{50} = 81.7; LT_{50} = 56.1) and *S. glaseri* caused only 46.6 ± 6.60% mortality even within 120 HAI (LC_{50} = 197.3; LT_{50} = 112.3).

In conclusion, the present study shows that the EPN fauna in the Ri-Bhoi District of Meghalaya is comprised of three nematode species, i. e., *H. indica, S. thermophilum* and *S. glaseri.* On the basis of bioefficacy assays the study proves that all the EPN species have considerable potentials to be used as biocontrol agents against the tested insect pests.

REFERENCES

Akhurst RJ (1980): Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *Journal of General Microbiology* 121: 303-309.

Akhurst RJ (1986): Controlling insects in soil with entomopathogenic nematodes. In: *Fundamental and applied aspects of invertebrate pathology*. Samson RA, Vlak M and Peters D (Eds.). Fourth International Colloquium of Invertebrate Pathology, Netherlands, pp. 265-267.

Akhurst RJ (1987): Use of starch gel electrophoresis in the taxonomy of the genus *Heterorhabditis* (Nematoda: Heterorhabditidae). *Nematologica* 24: 328-330.

Akhurst RJ (1990): Safety to invertebrate non-target organisms of nematodes used for biocontrol. In: *Safety of Microbial Control Agents*. Laird M, Lacey L and Davidson E (Eds.). CRC Press, Boca Raton, pp. 233-239.

Akhurst RJ and Brooks WM (1984): The distribution of entomophilic nematodes (Heterorhabditidae and Steinernematidae) in North Carolina. *Journal of Invertebrate Pathology* 44: 140-145.

Akhurst RJ and Bedding RA (1986): Natural occurrence of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in

soils in Australia. *Journal of Australia Entomological Society* 25: 241-244.

Alford DV (1990): A text book of agricultural entomology. Blackwell Science UK, pp. 255-265.

Amarasinghe LD, Hominick WM, Briscoe BR and Reid AP (1994): Occurrence and distribution of entomopathogenic nematodes in Sri Lanka. *Journal of Helminthology* 68: 277-286.

Ambika V (1995): Taxonomy, distribution and biology of native entomopathogenic nematodes. Dissertation submitted for M. Sc. (Agriculture) to the Tamil Nadu Agricultural University, Coimbatore.

Anis M, Shahina F, Reid AP and Rowe J (2002): *Steinernema asiaticum* sp. n. (Rhabditida: Steinernematidae) from Pakistan. *International Journal of Nematology* 12: 220-231.

Ansari, AM, Waeyenberge L and Moens M (2005): First record of *Steinernema glaseri* Steiner, 1929 (Rhabditida: Steinernematidae) from Belgium: a natural pathogen of *Hoplia philanthus* (Coleoptera: Scarabaeidae). *Nematology* 7: 953-956.

Artyukhovsky AK (1967): *Neoaplectana arenaria* nov. sp. Steinernematidae, Nematoda) inducing nematode disease in chafers of the Voronezh region. *Trudy Voronezhskogo Gosudarstvennogo Zapovednika* 15: 94-100.

Artyukhovsky AK, Kozodoi EM, Reid AP and Spiridonov SE (1997): Redescription of *Steinernema arenarium* (Artyukhovsky, 1967)

hopotype from Central Russia and a proposal for *S. anomalae* (Kozodoi, 1984) as a junior synonym. *Russian Journal of Nematology* 5: 31-37.

Banu GJ, Sosamma VK and Koshy PK (1998): Natural occurrence of an entomopathogenic nematode, *Heterorhabditis indicus* (Poinar, Karunakar and David, 1992) from Kerala India. In: *Nematology challenges and opportunities in 21st century,* Mehta UK (Ed.). *Proceedings of the Third International Symposium of Afro-Asian Society of Nematologists,* Coimbatore, India, pp. 271-280.

Banu JG, Nguyen KB and Rajendran G (2005): Occurrence and distribution of entomopathogenic nematodes in Kerala, India. *International Journal of Nematology* 15: 9-16.

Barbercheck ME and Kaya HK (1991): Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Environmental Entomology* 20: 582-589.

Barker CW and Barker GM (1998): Generalist entomopathogens as biological indicators of deforestation and agricultural land use impacts on Waikato soils. *Newzealand Journal of Ecology* 22:189-196.

Bathon H (1996): Impact of entomopathogenic nematodes on non-target hosts. *Biocontrol Science and Technology* 6: 421–434.

Beavers JB, Mc Coy CW and Kaplan DT (1983): Natural enemies of subterranean *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae in Florida. *Environmental Entomology* 12: 840–843.

Bedding RA (1981): Low cost *in vitro* mass production of *Neoplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. *Nematologica* 27: 109-114.

Bedding RA (1990): Logistics and strategies for introducing entomopathogenic nematode technology in developing countries. In: Entomopathogenic Nematodes for Biological Control, Gaugler R and Kaya HK (Eds.), CRC, Boca Raton, FL, pp. 233-248.

Bedding RA (1996): Future Directions for Entomopathogenic Nematodes. *Abstracts of the Second International Symposium on Entomopathogenic Nematodes and their Symbiotic Bacteria*, pp. 49-64.

Bedding RA and Akhurst RJ (1975): A simple technique for the detection of insect parasitic nematodes in soil. *Nematologica* 21: 109-110.

Bedding RA and Molyneux AS (1982): Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica* 28: 354-359.

Bednarek A and Mracek Z (1986): Compatibility of soil amendments with entomopathogenic nematodes. *Journal of Nematology* 29: 220-227.

Bhatnagar A, Shinde V and Bareth S (2004): Evaluation of entomopathogenic nematodes against whitegrub, *Maladera*

insanabilis Brenske. *International Journal of Pest Management* 50: 285-289.

Blackshaw RP (1988): A survey of insect parasitic nematodes in Northern Ireland. *Nematologica* 21: 109–110.

Boag B, Neilson R and Gordon SC (1992): Distribution and prevalence of the entomopathogenic nematode *Steinernema feltiae* in Scotland. *Annals of Applied Biology* 121: 355-360.

Boemare NE, Laumond C and Mauleon H (1996): The entomopathogenic nematode bacterium complex: Biology, life cycle and vertebrate safety. *Biocontrol Science and Technology* 6: 333-346.

Bovien P (1937): Some types of association between nematodes and insects. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening* 101: 1–114.

Brown IM and Gaugler R (1997): Temperature and humidity influence emergence and survival of entomopathogenic nematodes. *Nematologica* 43: 363-375.

Burman M, Abrahamsson K, Ascard J, Sjoberg A and Eriksson B (1986): Distribution of insect parasitic nematodes in Sweden. In: *Proceedings of the Fourth International Colloquium of Invertebrate Pathology.* Samson RA, Vlak JM and Peters D (Eds.), Society of Invertebrate Pathology, Wageningen, the Netherlands, p. 312.

Burnell AM and Stock SP (2000): *Heterorhabditis, Steinernema*, and their bacterial symbionts. Lethal pathogens of insects. *Nematology* 2: 31-42.

Cabanillas HE, Poinar GO and Raulston JR (1994): *Steinernema riobravis* sp. n. (Rhabditida: Steinernematidae) from Texas. *Fundamental and Applied Nematology* 17: 123-131.

Caicedo VAM and Bellotti AC (1996): Survey of native entomogenous nematodes associated with *Cyrtomenus bergi* Froeshner (Hemiptera: Cydnidae) in eight Colombian sites. *Revista Colombiana De Entomologica* 22: 19-24.

Campbell JF and Gaugler R (1993): Nictation behavior and its ecological implication in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behavior* 126: 155-169.

Caroli L, Glazer I and Gaugler R (1996): Entomopathogenic nematode infectivity assay: Comparison of penetration rate into different hosts. *Biocontrol Science and Technology* 6:227-233.

Chitwood BG (1933): On some nematodes of superfamily Rhabditoidea and their status as parasites of reptiles and amphibians. *Journal of Washington Academy Science* 23: 259-263.

Chitwood BG and Chitwood MB (1937): An introduction to Nematology. Monumental Printing Co., Baltimore, Maryland, pp. 53.

Choo HY, Kaya HK and Stock SP (1995): Isolation of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Korea. *Japanese Journal of Nematology* 25: 45-52.

Christie JR (1929): Some observation on sex in the Mermithidae. *Journal of Experimental Zoology* 53: 59-76.

Constant P, Marchay L, Fischer-Le Saux M, Briand-Panoma S and Mauleon H (1998): Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Guadeloupe islands. *Fundamental and Applied Nematology* 21: 667–672.

Courtney WD, Polley D and Miller VL (1955): TAF, an improved fixative in nematode technique. *Plant Disease Reporter* 39: 570-571.

Curran J (1989): Chromosome numbers of *Steinernema* and *Heterorhabditis* species. *Revue de Nematologie* 12: 145-147.

Curran J (1993): Post-application biology of entomopathogenic nematodes in soil. In: "*Nematodes and the biological control of insect pests,*" Bedding R, Akhurst R and Kaya HK (Eds.), CSIRO, East Melbourne, pp. 67-77.

Cutler CG and Stock SP (2003): *Steinernema websteri* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from China. *Nematologia Mediterranea* 31: 215-224.

Danilov LG (1987): Infestation and subsequent development of the nematode *Neoplectana carpocapsae* strain "Agriotos" in insects

under free contact between host and parasite. In: *"Helminthes of Insects"*, Sonin MD (Ed.), Amerind Publishing, New Delhi.

Desbach P and Rosen D (1991): Biological control by natural enemies. 2nd edition, Cambridge University Press, Cambridge New York, Melbourne, Sydney.

Dey S, Basu-Baul TS, Roy B and Dey D (1989): A new rapid method of air-drying for scanning electron microscopy using tetramethylsilane. *Journal of Microscopy* 156: 259-261.

Doucet MMA (1986): A new species of *Neoaplectana* Steiner, 1929 (Nematoda: Steinernematidae) from Cordoba, Argentina. *Revue de Nematologie* 9: 317-323.

Doucet MMA and Doucet ME (1990): *Steinernema ritteri* sp. n. (Nematoda: Steinernematidae) with a key to the species of the genus. *Nematologica* 36: 257-265.

Doucet MMA and Gabarra R (1994): On the occurrence of *Steinernema glaseri* (Steiner, 1929) (Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Heterorhabditidae) in Catalogne, Spain. *Fundamental and Applied Nematology* 17: 441-443.

Dutky SR, Thompson JV and Cantwell GE. (1964): A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology* 5: 417-422.

Ehlers RU, Deseo KV and Stackebrandt E (1991): Identification of *Steinernema* spp. (Nematoda) and their symbiotic bacteria

Xenorhabdus spp. from Italian and German soils. *Nematologica* 37: 360-366.

Elawad S, Ahmad W and Reid A (1997): *Steinernema abbasi* sp. n. (Nematoda: Steinernematidae) from the Sultanate of Oman. *Fundamental and Applied Nematology* 20: 433-442.

Fan X and Hominick WM (1991): Effects of low storage temperature on survival and infectivity of two *Steinernema* species (Nematoda: Steinernematidae). *Revue de Nematologie* 14: 407-412.

Figueroa W and Roman J (1990): Parasitism of entomophillic nematodes on the sugarcane root stalk borer, *Diaprepes abbreviatus* (L) (Coleoptera: Curcilionidae) larva. *Journal of Agriculture, University of Puerto Rico* 74: 197-202

Filipev IN (1934): Eine newe art der gattung *Neoaplectana* Steiner nebst Bemerkungen uber die systematishe sellung der letzteren. *Magasin de parasitologie de l'Institut zoologique des Sciences del' USSR.* IV: 229-240.

Fischer P and Fuhrer E (1990): Effect of soil acidity on the entomophillic nematode, *Steinernema kraussei* Steiner. *Biological Fertilizers of Soils* 9: 174-177.

Forschler BT and Nordin GL (1988): Comparative pathogenecity of selected entomogenous to the hardwood borers, *Prionoxystus robiniae* (Lepidoptera: Cosidae) and *Megacyllene robiniae* (Coleoptera: Cerambicidae). *Journal of Invertebrate Pathology* 52: 343-347.

Forschler BT and Gardner WA (1991): Field efficacy and persistence of entomogenous nematodes in the management of white grubs (Coleoptera: Scarabaediae) in turf and pasture. *Journal of Economic Entomology* 84: 1454-1459.

Ganguly S and Singh LK (2000): *Steinernema thermophilum* sp. n. (Rhabditida: Steinernematidae) from India. *International Journal of Nematology* 10: 183-191.

Ganguly S and Singh LK (2001): Optimum thermal requirements for infectivity and development of an indigenous entomopathogenic nematode, *Steinernema thermophilum* Ganguly and Singh, 2000. *Indian Journal of Nematology* 31: 148-452.

Ganguly S, Singh M, Lal M, Singh LK, Vyas RV and Patel DJ (2002): New record of an entomopathogenic nematode, *Steinernema riobrave* Cabanillas, Poinar and Raulston, 1994 from Gujarat, India. *Indian Journal of Nematology* 32: 223.

Gardner SL, Stock SP and Kaya HK (1994): A new species of *Heterorhabditis* from the Hawaiian Islands. *Journal of Parasitology* 80: 100-106.

Gaugler R and Kaya HK (1990): In: Entomopathogenic Nematodes for Biological Control, Gaugler R and Kaya HK (Eds.), CRC Press, Boca Raton, FL, pp. 365.

Gaugler R, Glazer I, Campbell JF and Liran N (1994): Laboratory and field evaluation of entomopathogenic nematodes genetically selected for improved host finding. *Journal of Invertebrate Pathology* 63: 68-73.

Georgis R and Poinar GO Jr. (1983): Effect of soil texture on the distribution of and infectivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). *Journal of Nematology* 15: 329-332.

Georgis R and Hom A (1992): Introduction of entomopathogenic nematode product in Latin America and Carribean. *Nematropica* 22: 81-98.

Georgis R and Manweiler SA. (1994): Entomopathogenic nematodes: a developing biological control technology. *Agricultural Zoology Reviews* 6: 63–94.

Ghode MK, Mishra RP, Swain PK, Nayak UK and Pawar AD (1988): Pathogenecity of *Steinernema feltiae* Weiser to *Heliothes armigera* Hubner. *Plant Protection Bulletin* 40: 7-8.

Glare TR, Jackson TA and Zimmermann G (1993): Occurrence of *Bacillus popilliae* and 2 nematode pathogens in populations of *Amphimallon solstitialis* (Coleoptera: Scarabaeidae) near Darmstadt, Germany. *Entomophaga* 38: 441–450.

Glaser RW and Fox H (1930): A nematode parasitic of the Japanese beetle (*Popillia japonica* Newman). *Science* 71: 16-17.

Glazer I (1992): Invasion rate as a measure of infectivity of steinernematid and heterorhabditid nematode to insects. *Journal of Parasitology* 59: 90-94.

Glazer I (2002): Survival biology - In: "*Entomopathogenic nematology*", Gaugler R (Ed.), CABI Publishing, Oxon, UK, 169-187.

Glazer I, Kozodoi E, Hashmi G and Gaugler R (1996): Biological characteristics of the entomopathogenic nematode, *Heterorhabditis* sp. IS-5: a heat tolerant isolate from Israel. *Nematologica* 42: 481-492.

Gokte-Narkhedkar N, Lavhe NV and Mayee CD (2001): Entomopathogenic nematodes- Potential agent against American bollworm of cotton (*Helicoverpa armigera*). *SAIC Newsletter* 11: 4.

Gomez KA and Gomez AA (1976): Statistical procedure for agricultural research with special emphasis on rice, IRRI, Los Banos, Phillipines, p. 386.

Gordon R, Chippett J and Tilley J (1996): Effects of 2 carbamates on infective juveniles of *Steinernema carpocapsae* all strain and *Steinernema feltiae* Umea strain. *Journal of Nematology* 28: 310-317.

Grewal PS, Gaugler R and Kaya HK (1993): Infectivity of entomopathogenic nematode *Steinernema scapterisci* (Nematoda: Steinernematidae). *Journal of Invertebrate Pathology* 62: 22-28.

Grewal PS, Selvan S and Gaugler R (1994): Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment and reproduction. *Journal of Thermal Biology* 19: 245–253.

Grewal PS, Ehlers RU and Shapiro-Ilan DI (2005): Nematodes as biological control. CABI Publishing, UK, pp. 505.

Griffin CT (1993): Temperature responses of entomopathogenic nematodes: Implications for the success of biological control programmes. *Nematodes and the biological control of insect pests.* Bedding R and Kaya HK (Eds.), East Melbourne, CSIRO. pp. 115-126.

Griffin CT, Downes MJ and Block W (1990): Tests of Antarctic soils for insect parasitic nematodes. *Antarctic Science* 2: 221-222.

Griffin CT, Moore JF and Downes MJ (1991): Occurrence of insect parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. *Nematologica* 37: 92–100.

Griffin CT, Joyce SA, Dix I, Burnell AM and Downes MJ (1994): Characterization of *Heterorhabditis* (Nematoda: Heterorhabditidae) from Ireland and Britain by molecular and cross-breeding techniques, and the occurrence of the genus in these islands. *Fundamental and Applied Nematology* 17: 245–253.

Griffin CT, Dix I, Joyce SA, Burnell AM and Downes MJ (1999): Isolation and characterization of *Heterorhabditis* spp. (Nematoda: Heterorhabditidae) from Hungary, Estonia, and Denmark. *Nematology* 1: 321–332.

Griffin CT, Chaerani R, Fallon D, Reid AP and Downes MJ (2000): Occurrence and distribution of the entomopathogenic nematodes

Steinernema spp. and *Heterorhabditis indica* in Indonesia. *Journal of Helminthology* 74: 143–150.

Gwynn RL and Richardson PN (1996): Incidence of entomopathogenic nematodes in soil samples collected from Scotland, England and Wales. *Fundamental and Applied Nematology* 19: 427–431.

Hara AH and Kaya HK (1982): Effect of selected insecticides and nematicides on the *in vitro* development of the entomogenous nematode *Neoplectana carpocapsae. Journal of Nematology* 14: 486-491.

Hara AH and Kaya HK (1983): Toxicity of selected organophosphate and carbamate pesticides to infective juveniles of the entomogenous nematode *Neoplectana carpocapsae* (Rhabditida: Steinernematidae). *Environmental Entomology* 12: 496-501.

Hara AH, Gaugler R, Kaya HK and LeBeck LM (1991): Natural populations of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) from the Hawaiian Islands. *Environmental Entomology* 17: 211–216.

Haukeland S (1993): Entomopathogenic nematodes found in Norway. *Norwegian Journal of Agricultural Science* 7: 17-27.

Hazir S, Kaya HK, Stock SP and Keskin N (2003): Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish Journal of Biology* 27: 181-202.

Hazir S, Stock SP and Keskin N (2003a): A new entomopathogenic nematode, *Steinernema anatoliense* n. sp. (Rhabditida: Steinernematidae), from Turkey. *Systematic Parasitology* 55: 211-220.

Hazir S, Keskin N, Stock SP, Kaya HK and Ozcan S (2003b): Diversity and distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Turkey. *Biodiversity and Conservation* 12: 375-386.

Hill DS (1987): Agricultural insect pests of temperate regions and their control. Cambridge University Press, UK.

Hominick WM and Tingley GA (1984): Mermithid nematodes and the control of insect vectors of human disease. *Biocontrol News and Information* 5: 7-20.

Hominick WM and Briscoe BR (1990): Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. *Parasitology* 100: 295–302.

Hominick WM and Reid AP (1990): Perspective on entomopathogenic nematology. In: "*Entomopathogenic nematodes in biological control*", Gaugler R and Kaya HK (Eds.), CRC Press, Boca Raton, FL, pp. 327-345.

Hominick WM, Reid AP and Briscoe BR (1995): Prevalence and habitat specificity of steinernematid and heterorhabditid nematodes

isolated during soil surveys of the UK and the Netherlands. *Journal of Helminthology* 69: 27-32.

Hominick WM, Reid AP, Bohan DA and Briscoe BR (1996): Entomopathogenic nematodes – biodiversity, geographical distribution and the convention on biological diversity. *Biocontrol Science and Technology* 6: 317–331.

Hussaini SS, Singh SP, Parthasarathy R and Shakeela V (2000): Storage effects on activity of native *Steinernema* and *Heterorhabditis* spp. *Indian Journal of Nematology* 30: 231-232.

Hussaini SS, Singh SP and Shakeela V (2001): Compatibility of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae: Rhabditida) with selected pesticides and their influence on some biological traits. *Entomology* 26: 37-44.

Hussaini SS, Singh SP and Nagesh M (2002): *In vitro* and field evaluation of some indigenous isolates of *Steinernema* sp. and *Heterorhabditis indica* against brinjal fruit and shoot borer, *Leucinodes orbonalis*. *Indian Journal of Nematology* 32: 63-65.

Hussaini SS, Singh SP and Shakeela V (2003): Variable efficacy of different formulations of entomopathogenic nematodes against black cutworm *Agrotis ipsilon* (Hufnagel) larvae. In: *Biological control of Lepidopteran Pests,* Tandon PL, Ballal CR, Jalali SK and Rabindra RJ (Eds.), Project Directorate of Biological Control, pp. 193-198.

Ignacimuthu S and Jayaraj S (2003): Biological control of insect pests. Phoenix Publication, New Delhi.

Iraki N, Salah N, Sansour MA, Segal D, Glazer I, Johnigk SA (2000): Isolation and characterization of two entomopathogenic nematode strains, *Heterorhabditis indica* from the West Bank, Palestinian Territories. *Journal of Applied Entomology* 124: 375–380.

Ishibashi N and Kondo E (1987): Dynamics of the entomogenous nematode *Steinernema feltiae* applied to soil with or without nematicide treatment. *Journal of Nematology* 19: 404-412.

Jain H, Reid AP and Hunt DJ (1997): *Steinernema ceratophorum* n. sp. (Nematoda: Steinernematidae) a new entomopathogenic from north east China. *Systematic Parasitology* 37: 115-125.

Josephrajkumar A and Sivakumar CV (1997): A survey for entomopathogenic nematodes in Kanyakumari district, Tamil Nadu, India. *Indian Journal of Entomology* 59: 45–50.

Joyce SA, Burnell AM and Powers TO (1994) Characterization of *Heterorhabditis* isolates by PCR amplification of segments of mtDNA and rDNA genes. *Journal of Nematology* 26: 260-270.

Karunakar G, Easwaramoorthy S and David H (1999): Susceptibility of nine Lepidopteran insects to *Steinernema glaseri, S. feltiae* and *Heterorhabditis indicus* infection. *International Journal of Nematology* 9: 68-71.

Karunakar G, Easwaramoorthy S and David H (2001): Influence of storage temperature, population density and duration of storage on the survival of three species of entomopathogenic nematodes. *Journal of Biological Control* 15: 183-188.

Kaushal KK, Renuka Rawat Nawed SH and Siyanand (2000): Survey of entomopathogenic nematodes in India. *Annals of Plant Protection Sciences* 8: 119-121.

Kaya HK (1990): Soil ecology. In: "*Entomopathogenic nematodes in biological control*", Gaugler R and Kaya HK (Eds.), CRC Press, Boca Raton, FL, pp. 93-115.

Kaya HK and Burlando TM (1989): Development of *Steinernema feltiae* (Rhabditida: Steinernematidae) in diseased insect hosts. *Journal of Invertebrate Pathology* 53: 164.

Kaya HK and Gaugler R (1993): Entomopathogenic nematodes. *Annual Review of Entomology* 38: 181–206.

Khan A, Brooks WW and Hirschmann H (1976): *Chromonema heliothidis* n. gens. n. sp. (Steinernematidae: Nematoda), a parasite of *Heliothis zea* (Noctuida: Lepidoptera) and other insects. *Journal of Nematology* 8: 159-169.

Kleespies R, Bathon H and Zimmermann G (1989): Investigation on the natural occurrence of entomopathogenic fungi and nematodes in different in surroundings of Dalmstadt, Germany. *Gesuude-Pflanzen* 41: 350-355.

Kondo E and Ishibashi N (1986): Infectivity and propagation of entomogenous nematodes, *Steinernema* spp., on the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Applied Entomological Zoology* 21: 95-108.

Kondo E and Ishibashi N (1988): Histological and SEM observations on succeeding growth of the entomopathogenic nematode *Steinernema feltiae* (Str. DD-136) in *Spodoptera litura* (Lepidoptera: Noctuidae) larvae. *Applied Entomological Zoology* 23: 88-96.

Koppenhofer AM, Kaya HK and Taormino SP (1995): Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. *Journal of Invertebrate Pathology* 65: 193-199.

Koppenhofer AM and Kaya HK (1999): Ecological characterization of *Steinernema rarum*. *Journal of Invertebrate Pathology* 73: 120–128.

Koppenhoffer AM, Ganguly S and Kaya HK (2000). Ecological characterization of *Steinernema monticolum*, a cold adapted entomopathogenic nematode from Korea. *Nematology* 2: 407–416.

Krishnayya PV and Grewal PS (2002): Effect of neem and selected fungicides on viability and virulence of the entomopathogenic nematode, *Steinernema feltiae*. *Biocontrol Science and Technology* 12: 259-266.

Kung SP, Gaugler R and Kaya HK (1990a): Soil type and entomopathogenic nematode persistence. *Journal of Invertebrate Pathology* 55: 401-406.

Kung SP, Gaugler R and Kaya HK (1990b): Influence of soil pH and oxygen on persistence of *Steinernema* spp. *Journal of Nematology* 22: 440-445.

Kung SP, Gaugler R and Kaya HK (1991): Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. *Journal of Invertebrate Pathology* 57: 242-249.

Lee SM, Lee DW and Choo HY (1996): Isolation of entomopathogenic nematodes and entomopathogenic fungi in the southern parts of Korea. *FRI - Journal of Forest Science* 53: 110-116.

Lewis EE, Gaugler R and Harrison R (1992): Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. *Parasitology* 105: 109-115.

Li XF and Wang GH (1989): Preliminary investigation on the distribution of Steinernematidae and Heterorhabditidae in Fujian, Guangdong and Hainan. *Chinese Journal of Zoology* 24: 1-4.

Liao CY, Tang LC, Pai CF, Hsiao WF, Briscoe BR and Hou RF (2001): A new isolate of the entomopathogenic nematode, *Steinernema abbasi* (Nematoda: Steinernematidae), from Taiwan. *Journal of Invertebrate Pathology* 77: 78–80.

Linstow O von (1905): Nematoda in the collection of the Colombo museum. *Spolia Zeylanica* 1: 91-104.

Liu J (1992): Taxonomic study of the genus *Steinernema* Travassos and *Heterorhabditis* Poinar. *Proceedings of 19th International Congress of Entomology,* Beijing China, pp. 318.

Liu J (1994): A new species of the genus *Heterorhabditis* from China (Rhabditida: Heterorhabditidae). *Acta Zootaxonomica Sinicia* 19: 268-272.

Liu J and Berry RE (1995): Natural distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Oregon soils. *Environmental Entomology* 24: 159–163.

Liu J and Berry RE (1996a): *Steinernema oregonensis* sp. n. (Rhabditida: Steinernematidae) from Oregon, USA. *Fundamental and Applied Nematology* 19: 375-380.

Liu J and Berry RE (1996b): *Heterorhabditis marelatus* n. sp. (Rhabditida: Heterorhabditidae) from Oregon. *Journal of Invertebrate Pathology* 67: 48-54.

Luc PV, Nguyen KB, Reid AP and Spiridonov SE (2000): *Steinernema tami* sp. n. (Rhabditida: Steinernematidae) from Cat Tien Forest, Vietnam. *Russian Journal of Nematology* 8: 33-43.

Mahar AN, Jan ND, Chachar QI, Markhand GS, Munir M and Mahar AQ (2005): Production and infectivity of some entomopathogenic

nematodes against larvae and pupae of cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae). *Journal of Entomology* 2: 86-91.

Mamiya Y (1988): *Steinernema kushidai* sp. n. (Nematoda: Steinernematidae) associated with scarabaeid beetle larvae from Shizuoca, Japan. *Applied Entomology and Zoology* 23: 313-320.

Mason JM and Hominick WM (1995): The effect of temperature on infection, development and reproduction of *Heterorhabditis*. *Journal of Helminthology* 69: 337-345.

Mason JM and Wright DJ (1997): Potential for the control of *Plutella xylostella* larvae with entomopathogenic nematodes. *Journal of Invertebrate Pathology* 70: 234-242.

Mason JM, Razak AR and Wright DJ (1996): The recovery of entomopathogenic nematodes from selected areas within Peninsular Malaysia. *Journal of Helminthology* 70: 303–307.

Mathur SB, Srivastava P and Srivastava KL (1971): Pathogenecity of DD-136 an Insect parasitic nematode to five pests of agricultural and horticultural importance. *Proceedings of National Academy of Sciences,* India 41: 325-327.

Mauleon H, Briand S, Laumond C and Bonifassi E (1993): Utilisation d'enzyme digestive peur l'étude du parasitisme des Steinernematidae et Heterorhabditidae envers les larves d' insects. *Fundamental and Applied Nematology* 16: 185–186.

Menti H, Wright DJ and Perry RN (1990): Infectivity of populations of the entomopathogenic nematodes *Steinenrnema feltiae* and *Heterorhabditis megidis* in relation to temperature, age and lipid content. *Nematology* 2: 515-521.

Menti H, Wright DJ and Perry RN (1997): Desiccation survival of populations of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* from Greece and the UK. *Journal of Helminthology* 71: 41-46.

Miduturi JS, Matata GJM, Waeyenberge L and Moens M (1996b): Naturally occurring entomopathogenic nematodes in the province of East Flanders, Belgium. *Nematologica Mediterranea* 24: 287-293.

Miduturi JS, Moens M, Hominick WM, Briscoe BR and Reid AP (1996a): Naturally occurring entomopathogenic nematodes in the province of West Flanders, Belgium. *Journal of Helminthology* 70: 319-327.

Miduturi JS, Waeyenberge L and Moens M (1997): Natural distribution of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) in Belgian soils. *Russian Journal of Nematology* 5: 55-65.

Molyneux AS (1985): Survival of infective juveniles of *Heterorhabditis* spp. and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. *Revue de Nématologie* 8: 165-170.

Molyneux AS, Bedding RA and Akhurst RJ (1983): Susceptibility of larvae of the sheep blowfly *Lucilia cuprina* to various *Heterorhabditis* spp., *Neoplectana* spp. and undescribed steinernematid (Nematoda). *Journal of Invertebrate Pathology* 42: 1-7.

Molyneux AS and Bedding RA (1984): Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the blowfly, *Lucilia cuprina*. *Nematologica* 30: 358-365.

Moyle PL and Kaya HK (1981): Dispersal and infectivity of the entomogenous nematode *Neoplectana carpocapsae* Weiser (Rhabditida: Steinernematidae) in sand. *Journal of Nematology* 13: 295-300.

Mracek Z (1980): The use of 'Galleria traps' for obtaining nematode parasites of insects in Czechoslovakia (Lepidoptera: Nematoda, Steinernematidae). *Acta Entomologica Bohemslovaca* 77: 378-382.

Mracek Z (1994): *Steinernema kraussei* (Steiner 1923) (Nematoda: Rhabditida: Steinernematidae): redescription of its topotype from Westphalia. *Folia Parasitologica* 41: 59-61.

Mracek Z, Gut J and Gerdin S (1982): *Neoaplectana bibionis* Bovien 1937, an obligate parasite of insects isolated from forest soil in Czechoslovakia. *Folia Parasitologica* 29: 139-145.

Mracek Z and Jenser G (1988): First report of entomogenous nematodes of the families Steinernematidae and Heterorhabditidae from Hungary. *Acta Phytopathologica et Entomologica Hungarica* 23: 153–156.

Mracek Z, Hanzal R and Kordrik D (1988): Sites of penetration of juvenile Steinernematids and Heterorhabditids (Nematoda) into the larvae of *Galleria mellonella* (Lepidoptera). *Journal of Invertebrate Pathology* 52: 477–478.

Mracek Z, Webster JM, Bures M and Kahounova L (1992): *Steinernema kraussei* (Steiner, 1923) (Nematoda: Rhabditida); rediscovery of its type locality in Germany. *Folia Parasitologica* 39: 181-199.

Mracek Z and Webster JM (1993): Survey of Steinernematidae and Heterorhabditidae (Rhabditida: Nematoda) in Western Canada. *Journal of Nematology* 25: 710–717.

Mracek Z, Hernandez EA and Boemare NE (1994): *Steinernema cubana* sp. n. (Nematoda: Rhabditida: Steinernematidae) and the preliminary characterization of its associated bacterium. *Journal of Invertebrate Pathology* 64: 123-129.

Mracek Z, Becvar S, Rezac P, Kindlmann P and Webster JM (1997): Canadian steinernematid isolates and their infectivity under cold conditions to greater wax moth, *Galleria mellonella,* larvae. *Biological Control* 8: 160-164.

Mracek Z, Becvar S and Kindlmann P (1999): Survey of entomopathogenic nematodes from the families Steinernematidae and

Heterorhabditidae (Nematoda: Rhabditida) in the Czech Republic. *Folia Parasitologica* 46: 145–148.

Mracek Z and Becvar S (2000): Insect aggregations and entomopathogenic nematode occurrence. *Nematology* 2: 297–301.

Mracek Z and Sturhan D (2000): Epizootic of the entomopathogenic nematode *Steinernema intermedium* (Poinar) in an aggregation of the bibionid fly, *Bibio marci* L. *Journal of Invertebrate Pathology* 76: 149-50.

Mracek Z, Sturhan D and Reid AP (2003): *Steinernema weiseri* n. sp. (Rhabditida, Steinernematidae), a new entomopathogenic nematode from Europe. *Systematic Parasitologica* 56: 37–47.

Mracek Z, Becvar S, Kindlmann P and Jersakova J (2005): Habitat preference for entomopathogenic nematodes, their insect hosts and new faunistic records for the Czech Republic. *Biological Control* 34: 27–37.

Narayanan K and Gopalakrishna C (2003): Evaluation of entomopathogenic nematode, *Steinernema feltiae* against field population of mustard sawfly, *Athalia lugens proxima* (Klug) on radish. *Indian Journal of Experimental Biology* 41: 376-378.

Navon A and Ascher KRS (2000): Bioassays of entomopathogenic microbes and nematodes. CABI Publishing, UK, p. 336.

Nguyen KB and Smart GC Jr. (1990): *Steinernema scapterisci* sp. n. (Steinernematidae: Nematoda). *Journal of Nematology* 22: 187-199.

Nguyen KB and Smart GC Jr. (1992): *Steinernema neocurtillis* sp. n. (Rhabditida: Steinernematidae) and a key to species of the genus *Steinernema*. *Journal of Nematology* 24: 160-169.

Nguyen KB and Smart GC Jr. (1994): *Neosteinernema longicurvicauda* n. gen. n. sp. (Rhabditida: Steinernematidae), a parasite of the termite *Reticulitermes flavipes* (Koller). 1994. *Journal of Nematology* 26: 162-174.

Nguyen KB and Duncan LW (2002): *Steinernema diprepesi* n. sp. (Rhabditida: Steinernematidae), a parasite of the citrus weevil *Diaprepes abbreviatus* (L) (Coleoptera: Curculionidae). *Journal of Nematology* 34: 159-170.

Nguyen KB, Tesfamariam M, Gozel U, Gaugler R and Adams BJ (2004a): *Steinernema yirgalemense* n. sp. (Rhabditida: Steinernematidae) from Ethiopia. *Nematology* 6: 839–856.

Nguyen KB, Shapiro-Ilan DI, Stuart RJ, McCoy CW, Rosalind RJ and Adams BJ (2004b): *Heterorhabditis mexicana* n. sp. (Rhabditida: Heterorhabditidae) from Tamaulipas, Mexico, and morphological studies of the bursa of *Heterorhabditis* spp. *Nematology* 6: 231-244.

Nguyen KB, Gozel U, Koppenhofer HS and Adams BJ (2006a): *Heterorhabditis floridensis* n. sp. (Rhabditida: Heterorhabditidae) from Florida. *Zootaxa* 1177: 1–19.

Nguyen KB, Malan AP and Gozel U (2006b): *Steinernema khoisanae* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 8: 157-175.

Nishimatsu T and Jackson JJ (1998): Interaction of insecticides, entomopathogenic nematodes and larvae of the western corn rootworm (Coleoptera: Chrysomellidae). *Journal of Economic Entomology* 91: 410-418.

Orley L (1880): Az angillulidak maganrajza. A kir. M. termeszettudom. Tersulat altal a bugatdijjal jutalmazott palyamii. *Termeszetr. Fuz.* 4: 16-50.

Ozer N, Keskin N and Kirbas Z (1995): Occurrence of entomopathogenic nematodes (Steinernematidae: Heterorhabditidae) in Turkey. *Nematologica* 41: 639–640.

Parihar A, Siddique AU, Yadav YS and Ram B (2002): Occurrence of entomopathogenic nematodes in Rajasthan. Proceedings of *National Symposium on Biodiversity and Management of Nematodes in Cropping Systems for Sustainable Agriculture,* ARS, Durgapura (Jaipur), Rajasthan, pp. 80.

Pathak KA, Thakur NSA, Rao KR and Shylesha AN (2001): Insect pests of crops and their management. In: *Steps towards modernization of Agriculture in NEH region,* Verma ND and Bhatt BP (Eds.), pp. 121-159.

Petersen JJ (1972): Factors affecting the sex ratios of mermithid parasites of mosquitoes. *Journal of Nematology* 4: 83-87.

Phan KL, Nguyen NC and Moens M (2001a): *Steinrnema sangi* sp. n. (Rhabditida: Steinernematidae) from Vietnam. *Russian Journal of Nematology* 9: 1-7.

Phan KL, Nguyen NC and Moens M (2001b): *Steinernema loci* sp. n. and *Steinernema thanhi* sp. n. (Rhabditida: Steinernematidae) from Vietnam. *Nematology* 3: 503-514.

Phan KL, Subbotin SA, Nguyen NC and Moens M (2003): *Heterorhabditis baujardi* sp. n. (Rhabditida: Heterorhabditidae) from Vietnam with morphometric data for *H. indica* populations. *Nematology* 5: 367-382.

Phan KL, Subbotin SA, Waeyenberge L and Moens M (2005): A new entomopathogenic nematode, *Steinernema robustispiculum* n. sp. (Rhabditida: Steinernematidae), from Chumomray National Park in Vietnam. *Systematic Parasitologica* 60: 23-32.

Pino FG, Palomo A and Del PFG (1996): Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Spanish soils. *Journal of Invertebrate Pathology* 68: 84-90.

Poinar GO Jr. (1976): Description and biology of a new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen. n. sp. (Rhabditida: Heterorhabditidae n. fam.). *Nematologica* 21: 463-470.

Poinar GO Jr. (1979): Nematodes for Biological Control of Insects. CRC Press, Boca Raton, Florida.

Poinar GO Jr. (1983): The natural history of nematodes. Prentice Hall, Englewood Cliffs.

Poinar GO Jr. (1985): *Neoaplectana intermedia* sp. n. (Steinernematidae: Nematoda) from South Carolina. *Revue de Nematology* 8: 321-327.

Poinar GO Jr. (1986): Recognition of *Neoaplectana* species (Steinernematidae: Rhabditida). *Proceedings of the Helminthological Society of Washington* 53: 121-129.

Poinar GO Jr. (1990): Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: *Entomopathogenic nematodes in biological control,* Gaugler R and Kaya HK (Eds.), CRC Press, Boca Raton, Florida, pp. 23-60.

Poinar GO Jr. and Veramchuk GV (1970): A new strain of nematodes pathogenic to insects and the geographical distribution of *Neoplectana carpocapsae* Weiser (Rhabditida: Steinernematidae). *Zoological Zhurnal* 49: 966-969.

Poinar GO Jr., Thomas GM and Hess R (1977): Characteristics of the specific bacterium associated with *Heterorhabditis bacteriophora* (Heterorhabditis: Rhabditida). *Nematologica* 23: 97-102.

Poinar GO Jr., Jackson T and Klein M (1987): *Heterorhabditis megidis* sp. n. (Heterorhabditidae: Rhabditida) parasitic in the Japanese beetle,

Popillia japonica (Scarabaeidae: Coleoptera), in Ohio. *Proceedings of the Helminthological Society of Washington* 54: 53-59.

Poinar GO Jr. and Georgis R (1990): Description and field application of the HP88 strain of *Heterorhabditis bacteriophora. Revue de Neamatologie* 13: 387-393.

Poinar GO, Karunakar GK and David H (1992): *Heterorhabditis indicus* n. sp. (Rhabditida: Nematoda) from India: separation of *Heterorhabditis* spp. by infective juveniles. *Fundamental and Applied Nematology* 15: 467-472.

Popiel I, Grove DL and Friedman MJ (1989) Infective juvenile formation in the insect parasitic nematode *Steinernema feltiae. Parasitology* 99: 77-81.

Prasad GS, Ranganath HR and Singh PK (2001a): Occurrence of the entomopathogenic nematode in parts of South Andamans. *Current Science* 80: 501-502.

Prasad GS, Singh PK and Ranganath HR (2001b): Population fluctuation of entomopathogenic nematode, *Heterorhabditis* spp. in South Andaman as influenced by weather parameters. *Current Science* 80: 923-924.

Puza V and Mracek Z (2004). Seasonal population dynamic of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* and their insect hosts, with comments on the winter

period. *Society for Invertebrate Pathology,* 37th Annual Meeting in Helsinki, Finland 1-6 August 2004.

Qiu L, Fang Y, Zhou Y, Pang Y and Nguyen KB (2004): *Steinernema guangdongense* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from southern China with a note on *S. serratum* (*nomen nudum*). *Zootaxa* 704: 1–20

Qiu L, Yan X, Zhou Y, Nguyen KB and Pang Y (2005a): *Steinernema aciari* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from Guangdong, China. *Journal of Invertebrate Pathology* 88: 58-69.

Qiu L, Hu X, Zhou Y, Mei S, Nguyen KB and Pang Y (2005b): *Steinernema akhursti* sp. n. (Nematoda: Steinernematidae) from Yunan, China. *Journal of Invertebrate Pathology* 90: 151-160.

Qiu L, Hu X, Zhou Y, Pang Y and Nguyen KB (2005c): *Steinernema beddingi* n. sp. (Nematoda: Steinernematidae), a new entomopathogenic nematodes from Yunan, China. *Nematology* 7: 737-749.

Rajkumar M, Parihar A and Siddiqui AU (2001): Studies on entomopathogenic nematodes of Udaipur. *Proceedings of National Congress of Centenary of Nematology in India: Appraisal and Future Plans.* IARI, New Delhi, pp.118.

Ramadhane AM and Ihsan SI (1999): Biological characteristics related to the life cycle of cabbage butterfly, *Pieris brassicae* L. (Lepidoptera:

Pieridae) and associated endoparasitoids. *Arab Journal of Plant Protection* 17: 45-48.

Rao VP and Manjunath TM (1966): DD-136 nematode that can kill many insect pests. *Indian Farming* 16: 43-44.

Roman J and Figueroa W (1994): *Steinernema puertoricensis* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Puerto Rico. *Journal of Agriculture, University of Puerto Rico* 78: 167-175.

Rosa JS, Bonifassi E, Amaral J, Lacey LA, Simoes N and Laumond C (2000): Natural occurrence of entomopathogenic nematodes (Rhabditida: *Steinernema, Heterorhabditis*) in the Azores. *Journal of Nematology* 32: 215–222.

Rovesti L, Heinzpeter EW Tagliente F and Deseo KV (1988): Compatibility of pesticides with the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae). *Nematology* 34: 462-476.

Rovesti L and Deseo KV (1989): Effect of neem kernel extract on steinernematid and heterorhabditid nematodes. *Nematologica* 35: 493-496.

Rovesti L and Deseo KV (1990): Compatibility of chemical pesticides with the entomopathogenic nematodes, *Steinernema carpocapsae* Weiser and *S. feltiae* Filipjev (Nematoda: Steinernematidae). *Nematology* 36: 237-245.

Rueda LM, Osawaru SO, Georgei LL and Harrison RE (1993): Natural occurrence of entomogenous nematodes in Tennessee nursery soils. *Journal of Nematology* 25: 181-188.

Sandner H and Stanuzek S (1971): Comparative research on the effectiveness and production of *Neoplectana carpocapsae. Zesty problemowe Postepow Nauk rolniczych.* 121: 209-226.

Sandner H and Bednarek A (1987): Entomophilic nematodes in the Swietokrzyski National Park. *Fragmenta Faunistica* 31: 3-9.

Schroeder WJ and Beavers JB (1987): Movement of entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae in soil. *Journal of Nematology* 19: 257-259.

Seinhorst JW (1959): A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* 4: 117–128.

Selvan S, Campbell JF and Gaugler R (1993): Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *Journal of Invertebrate Pathology* 62: 278-284.

Shahina F, Anis M, Reid AP, Rowe J and Maqbool (2001): *Steinernema pakistanense* sp. n. (Rhabditida: Steinernematidae) from Pakistan. *International Journal of Nematology* 11: 124-133.

Shamseldean MM and Abd-Elgawad MM (1994) : Natural occurrence of insect pathogenic nematodes (Rhabditida : Heterorhabditidae) in Egyptian soils. *Afro-Asian Journal of Nematology* 4 : 151-154.

Shamseldean MM, Abou El-Sooud A.B, Abd-Elgawad MM and Saleh MM (1996) : Identification of a new heterorhabditid species from Egypt, *Heterorhabditis taysearae,* n. sp. (Rhabditida: Heterorhabtidae). *Egyptian Journal of Biological Pest Control* 6: 129-138.

Shannag HK, Webb SE and Capinera JL (1994): Entomopathogenic effect on pickleworm (Lepidoptera: Pyralidae) under laboratory and field conditions. *Journal of Economic Entomology* 87: 1205-1212.

Shannag HK and Capinera JL (1995): Evaluation of entomopathogenic nematode species for the control of melon worm (Lepidoptera: Pyralidae). *Environmental Entomology* 24: 143-148.

Shen CP (1992): Description of an entomopathogenic nematode, *Steinernema longicaudum* sp. nov. and its application. In: *Proceedings of the 19th International Congress of Entomology,* Beijing, China, pp. 220-231.

Shylesha AN (2003): Potential of entomopathogenic nematodes for insect control in North Eastern hill region. In: *"Current status of research on entomopathogenic nematodes in India",* Hussaini SS, Rabindra RJ and Nagesh M (Eds.), pp. 127-134.

Singh J and Bardhan V (1974): Effectiveness of DD 136 an entomophilic nematode against insect pests of agriculture importance. *Current Science* 43: 662.

Singh M, Sharma SB and Ganga Rao GV (1992): Occurrence of entomopathogenic nematodes at ICRISAT centre. *International Arachis Newsletter* 12: 15-16.

Singh SP (1977): Isolation of entomophilic nematodes from potato cutworms. *Current Science* 46: 454-455.

Singh SP (1997): Technology for Production of Natural Enemies. Project Directorate of Biological Control, Bangalore India, *Technical Bulletin No.* 4: 221.

Smits PH (1996): Post-application persistence of entomopathogenic nematodes. *Biocontrol Science and Technology* 6: 379-387.

Singh SP (1994): *Technology for Production of Natural Enemies.* Project Directorate of Biological Control, Bangalore India, *Technical Bulletin No. 4,* pp. 221.

Sivakumar CV, Jayaraj S and Subramanian S (1989): Observation on Indian population of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar, 1976. *Journal of Biological Control* 2: 112-113.

Sivakumar M, Poornima K, Mani MP and Uma Maheshwari R (2002): Occurrence of beneficial nematodes in the western ghats of Tamil Nadu. In: Proceedings of National Symposium on *"Biodiversity and Management of Nematodes in Cropping Systems for Sustainable Agriculture"*, ARS, Durgapur, Jaipur, p. 79.

Sosamma VK (2003): Utilisation of entomopathogenic nematodes in plantation crops. In: *"Current status of research on entomopathogenic nematodes in India"*, Hussaini SS, Rabindra RJ and Nagesh M (Eds.), pp. 109-120.

Sosamma VK, Jaijo MJ and Rasmi B (2002): Occurrence of entomogenous nematodes and an entomopathogenic fungus, *Metarhizium flavoviride* associated with rhinoceros beetle, a pest of coconut. *Indian Journal of Nematology* 32: 78-79.

Spiridonov SE, Krasomil-Osterfeld K and Moens M (2004): *Steinernema jollieti* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from the American Midwest. *Russian Journal of Nematology* 12: 85.

Stanuszek S (1974) *Neoplectana feltiae, Pieridarum* n. ecotype (Nematoda: Rhabditidae, Steinernematidae) a parasite of *Pieris brassicae* L. and *Mamestra brassicae* L. in Poland. Morphology and Biology. *Zeszyty Problemowe Postepow Nauk Rolniczch* 154: 361-367.

Steiner G (1923): *Aplectana kraussei* n. sp., eine in der Blattwespe *Lyda* sp. parasitierende Nemodenform, nebst Bemerkungen uÈ ber das Seitenorgan der parasitischen Nematoden. *Zentralblatt fuÈ r Bakteriologie, Parasitenkunde, Infektions, Krankheiten und Hygiene, Abteilung II* 59: 14-18.

Steiner G (1929): *Neoaplectana glaseri*, n.g.n.sp. (Oxyuridae), a new endemic parasite of the Japanese beetle (*Popillia japonica*, Newm.). *Journal of Washington Academy Science* 19: 436-440.

Steiner WA (1994) Distribution of entomopathogenic nematodes in the Swiss Alps. In: *COST 812 Biotechnology:Genetics of Entomopathogenic Nematode- Bacterium Complexes, Proceedings of Symposium and Workshop,* St Patrick' s College, Maynooth, Co. Kildare, Ireland (Burnell AM, Ehlers RU and Masson JP (Eds.). European Commission, DG XII, Luxembourg, p. 218.

Steiner WA (1996): Distribution of entomopathogenic nematodes in the Swiss Alps. *Suisse Journal de Zoologie* 103: 439–452.

Stock SP (1993): A new species of the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) parasitic of *Graphognathus* sp. larvae (Coleoptera: Curculionidae) from Argentina. *Research and Reviews in Parasitology* 53: 103-107.

Stock SP (1995): Natural Populations of entomopathogenic nematodes from the Pampean Region of Argentina. *Nematropica* 25: 143-148.

Stock SP, Strong DR and Gardner SL (1996): Identification of *Heterorhabditis* (Nematoda: Heterorhabditidae) from California with a new species isolated from the larvae of the ghost moth *Hepialis californicus* (Lepidoptera: Hepialidae) from the Bodega Bay Natural Preserve. *Fundamental and Applied Nematology* 19: 585-592.

Stock SP, Choo HY and Kaya HK (1997): An entomopathogenic nematode, *Steinernema monticolum* sp. n. (Rhabditida: Steinernematidae) from Korea with a key to other species. *Nematologica* 43: 15-29.

Stock SP, Somsook V and Reid AP (1998): *Steinernema siamkayai* sp. n. (Rhabditida: Steinernematidae), an entomopathogenic nematode from Thailand. *Systematic Parasitology* 41: 105-113.

Stock SP, Pryor BM and Kaya HK (1999): Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California. *Biodiversity and Conservation* 8: 535-549.

Stock SP, Heng J, Hunt DJ, Reid AP, Shen X and Choo HY (2000): Redescription of *Steinernema longicaudum* Shen and Wang (Nematoda: Steinernematidae). Geographic distribution and phenotypic variation between allopatric populations. *Journal of Helminthology* 75: 81-92.

Stock SP, Burnell AM and Griffin CT (2002): Morphological characterization of three isolates of *Heterorhabditis* Poinar, 1976 from the "Irish group" (Nematoda: Rhabditida: Heterorhabditidae) and additional evidence supporting their recognition as a distinct species, *H. downesi* n. sp. *Systematic Parasitology* 51: 95-106.

Stock SP and Koppenhofer AM (2003): *Steinernema scarabaei* n. sp. (Rhabditida: Steinernematidae), a natural pathogen of scarab beetle

larvae (Coleoptera: Scarabaeidae) from New Jersey, USA. *Nemtaology* 5: 191–204.

Stock SP, Griffin CT and Chaenari R (2004): Morphological and molecular characterization of *Steinernema hermaphroditum* sp. n. (Nematoda: Steinernematidae), an entomopathogenic nematode from Indonesia, and its phylogenetic relationship with other closely related taxa. *Nematology* 6: 401-412.

Strong DR (2002): Population of entomopathogenic nematodes in foodwebs. In: *Entomopathogenic Nematology,* Gaugler R (Ed.), Oxon, UK, CABI Publishing, pp. 225-240.

Stuart RJ and Gaugler R (1994): Patchiness in populations of entomopathogenic nematodes. *Journal of Invertebrate Pathology* 64: 39-45.

Sturhan D (1991): Studies on the natural occurrence of *Steinernema* and *Heterorhabditis* in soil in West Germany. *Nematologica* 36: 394.

Sturhan D (1995): Untersuchungen über sympatrisches Vorkommen entomopathogener Nematoden. *Nachrichtenbl.Deut. Pflanzenschutz* 47: 54.

Sturhan D (1997): Untersuchungen zum Artenspektrum entomopathogener Nematoden in verschiedenen Biotopen. Biologische Vielfalt in Ökosystemen - Konflikt zwischen Nutzung und Erhaltung. L. u. F. Bundesministerium für Ernährung. Bonn, Köllen Druck and Verlag GmbH: 72.

Sturhan D (1999): Prevalence and habitat specificity of entomopathogenic nematodes in Germany. *Proceedings of a workshop held at Todi, Perugia, Italy, 16 to 29 May 1995.* Luxembourg: European Commission, COST 819, pp. 123–132.

Sturhan D and Liskova M (1999): Occurrence and distribution of entomopathogenic nematodes in the Slovak Republic. *Nematology* 1: 273–277.

Sturhan D, Spiridonov S and Mracek Z (2005): *Steinernema silvaticum* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Europe. *Nematology* 7:227-241.

Sundarababu R, Menon PPV and Vadivelu S (1984): Preliminary tests with DD-136 for the control of potato chafer grub, *Anomala* sp. *Indian Journal of Nematology* 14: 187-188.

Surrey MR and Wharton DA (1995): Dessication survival of the infective larvae of the insect parasitic nematode, *Heterorhabditis zealandica* Poinar. *International Journal of Parasitology* 25: 749-752.

Subramanian S and Sundarababu R (2002): Foliar persistence of entomopathogenic nematode. In: *Proceedings of National Symposium on "Biodiversity and Mangement of Nematodes in Cropping Systems for Sustainable Agriculture"*, ARS, Durgapura, Jaipur, p. 79.

Tallosi B, Selculic R and Keresi T (1993): Investigations on entomopathogenic nematodes in the Vojvodina and the possibility of

their use in the control of some agricultural pests. *Zastita Bilja* 44: 213-219.

Tallosi B, Peters A and Ehlers R (1995): *Steinernema bicornutum* sp. n. (Rhabditida: Steinernematidae) from Vojvodina, Yugoslavia. *Russian Journal of Nematology* 3: 71-80.

Tangchitsomkid N and Sontirat S (1998): Occurrence of entomopathogenic nematodes in Thailand. *The Kasetsart Journal: Natural Sciences* 32: 347–354.

Tarasco E and Triggiani O (1996): Survey of *Steinernema* and *Heterorhabditis* (Rhabditida: Heterorhabditidae, Steinernematidae). *Entomologica* 31: 117-123.

Tingley GA and Anderson RM (1986): Environmental sex determination and density dependent population regulation in the entomogenous nematode *Romanomermis culicivorax. Parasitology* 92: 431-449.

Toledo J, Gurgua JL, Liedo P, Ibarra JE and Oropeza A (2001): Parasitismo de larvas y pupas de la mosca mexicana de la fruta, Anastrephaludens (Loew) (Diptera: Tephritidae) por el nemátodo *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae). *Vedalia* 8: 27-36.

Travassos L (1920): Contribuicoes para o conhecimento da fauna helmintologica brasileira. Oxyascaridae n. fam. *Arch Esc Sup Agric Medic Vet* 4: 15.

Travassos L (1927): Sobre O Geneva Oxystomatium. *Boletium Biologico (Sau Paulo)* 5: 20-21.

Triggiani 0 and Poinar G0 Jr. (1976): Infection of adult Lepidoptera by *Neoplectana carpocapsae* (Nematoda). *Journal of Invertebrate Pathology* 27: 413-414.

Triggiani O, Mracek Z, Reid A (2004): *Steinernema apuliae* sp. n. (Rhabditida: Steinernematidae): a new entomopathogenic nematode from southern Italy. *Zootaxa* 460: 1-12.

Uribe-Lorio L, Mora M and Stock SP (2005): First record of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Costa Rica. *Journal of Invertebrate Pathology* 88: 226-231.

Vyas RV, Pharindera Y, Choudhary R, Patel NS and Yadav P (1998): Natural incidence of entomopathogenic nematodes in central north Gujarat. In: *Proceedings of National Symposium on Rational Approaches in Nematode Management for Sustainable Agriculture,* Dhawan S.C. (Ed.), Anand, India, 23–25th November, 1998. Nematological Society of India, New Delhi, India, pp. 86–88.

Waturu CN, Hunt DJ and Reid AP (1997): *Steinernema karii* sp n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from Kenya. *International Journal of Nematology* 7: 68-75.

Weiser J (1955): *Neoaplectana carpocapsae* n. sp. (Anguillulata, Steinernematidae) novy cizopasnik housenik obalece jablecneÂho,

Carpocapsa pomonella L. *Vestnik CeskoslovenskeÂ ZoologickeÂ Spolecnosti* 19: 44-52.

Wharton DA (1995): Cold tolerance strategies in nematodes. *Biological Review* 70: 161-185.

White GF (1927): A method for obtaining infective nematode larvae from cultures. *Science* 66: 302-303.

Womersley CZ (1987): A re-evaluation of strategies employed by nematode anhydrobiotes in relation to their natural environment. In: *Vistas on Nematology: A commemoration for the 25th Anniversary of the Society of Nematologist,* Veech JA and Dickinson DW (Eds.), Hyattsville, Maryland, pp.165–173.

Womersley CZ (1990): Critical aspects of entomopathogenic nematode physiology. *Proc. Vth Int. Coll. Invertebrate Pathology Microbial Control.* P. Society for Invertebrate. Adelaide, Australia, p. 222.

Womersley CZ (1993): Factors affecting physiological fitness and modes of survival employed by dauer juveniles and their relationship to pathogenicity. In: *Nematodes and the biological control of insect pests,* Bedding R, Akhurst R and Kaya H (Eds.). East Melbourne, CSIRO, pp. 79-88.

Woodring JL and Kaya HK (1988): Steinernematid and Heterorhabditid nematodes. A hand-book of techniques. Southern Cooperative Series Bulletin. 331. Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

Wout WM (1980): Biology, life cycle and redescription of *Neoaplectana bibionis* Bovien, 1937 (Nematoda: Steinernematidae). *Journal of Nematology* 12: 62-72.

Wout WM, Mracek Z, Gerdin S and Bedding RA (1982): *Neoaplectana* Steiner, 1929 a junior synonym of *Steinernema* Travassos, 1927 (Nematoda: Rhabditida). *Systematic Parasitology* 4: 147-151.

Xu Z, Wang G. and Li X (1991): A new species of the genus *Steinernema* (Rhabditida: Steinernematidae). *Zoological Research* 12: 17-20.

Yadava CP and Rao YS (1970): On the effectiveness of the entomophilic nematode DD-136 In the biological control of Insect pests of rice. *Oryza* 7: 131-136.

Yoshida M, Reid AP, Briscoe BR and Hominick WM (1998): Survey of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Japan. *Fundamental and Applied Nematology* 21: 185-198.

Yoshida M (2004): *Steinernema litorale* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Japan. *Nematology* 6: 819-838.

Zervos S, Johnson SC and Webster JM (1991): Effect of temperature and inoculum size on reproduction and development *Heterorhabditis heliothidis* and *Steinernema glaseri* in *Galleria mellonella. Canadian Journal of Zoology* 69: 1261-1264.

Zhang L, Shono T, Yamanaka S and Tanabe H (1994): Effects of insecticides on the entomopathogenic nematode *Steinernema carpocapsae*. *Applied Entomology and Zoology* 29: 539-547.

Zimmerman RJ and Cranshaw WS (1990): Compatibility of three entomogenous nematodes (Rhabditida) in aqueous solution of pesticides used in turf grass maintenance. *Journal of Economic Entomology* 83: 97-100.

BIO-DATA

Name	:	LALRAMLIANA
Father's name	:	LALRINAWMA
Present address	:	C/o Dr. Arun Kumar Yadav Department of Zoology North-Eastern Hill University Shillong 793 022
Permanent Address	:	C-94, Chanmari West, Aizawl, Mizoram
E-mail	:	lrl_zoo@yahoo.co.in
Date of Birth	:	14 – 10 – 1975

Academic qualifications

Exams. passed	Year	Division	Board/University
H.S.L.C	1991	I	MBSE
P.U (Sc.)	1994	II	NEHU
B.Sc.	1997	I	NEHU
M.Sc.	2000	I	NEHU
Ph. D.	2007 (Submission of Thesis)		NEHU

Other qualification	:	NET (CSIR-UGC) in Life Sciences
Fellowship awarded	:	JRF- 2 years and SRF - 2 years (UGC, N. Delhi)

Experience:

1. Completed two (2) months training course on *"Biological Control of Crop Pests in Different Cropping Systems."* at Project Directorate of Biological Control, Bangalore.
2. Completed six (6) days refresher course on *"Biological Control of Crop Pests in Different Cropping Systems."* at Project Directorate of Biological Control, Bangalore.

Conference/Workshop attended and presented research findings:

1. **Lalramliana,** Yadav AK, Grewal PS and Shylesha AN (2003): Evaluation of optimal temperature and concentration of infective juveniles for the bioefficacy test of indigenously isolated entomopathogenic nematodes in Meghalaya, India. In: *Third International Symposium on Entomopathogenic nematodes and their symbiotic bacteria.* Arden Shisler Conference Center, Ohio State University, Wooster, Ohio, USA on Sept. 3-7, 2003.
2. Yadav AK, **Lalramliana**, Grewal PS and Shylesha AN (2003): A survey of entomopathogenic nematodes in Meghalaya, India. In: *Third International Symposium on Entomopathogenic nematodes and their symbiotic bacteria.* Arden Shisler Conference Center, Ohio State University, Wooster, Ohio, USA on Sept. 3-7, 2003.
3. **Lalramliana**, Yadav AK and Shylesha AN (2004): Influence of temperature and relative humidity on the emergence of infective juveniles of *Heterorhabditis* sp. - a locally isolated entomopathogenic nematode in Meghalaya, India. In: *3rd Global Meet on Parasitic Diseases,* Bangalore on Jan. 12-16, 2004.
4. **Lalramliana**, Yadav AK and Shylesha AN (2005): Effect of different soil moistures level on the pathogenicity of locally isolated entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Meghalaya, India. In: *17th National congress of Parasitology,* Dibrugarh, Assam on Oct. 24-26, 2005.
5. **Lalramliana**, Yadav AK and Shylesha AN (2006): Biocontrol potentials of entomopathogenic nematodes against colocasia corm borer, *Haplosonyx chalybaeus* Hope (Coleoptera: Chrysomellidae). In: *Regional Symposium on Current research thrust in animal sciences.* NEHU, Shillong on Mar. 24-25, 2006.
6. **Lalramliana**, Yadav AK and Shylesha AN (2007): Infectivity and production of three locally isolated entomopathogenic nematodes in brinjal fruit and shoot borer, *Leucinodes orbonalis* Green (Lepidoptera: Pyralidae). In: *Regional Symposium on Current research thrust in animal sciences: Interface with end use researchers and stakeholders.* NEHU, Shillong on Mar. 15-16, 2007.