

# **STUDIES ON ENTOMOPATHOGENIC NEMATODES**

*By*

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## *Certificate*

**Mr.SREERAM NANDA KRISHNA** has satisfactorily prosecuted the course of research and that the thesis entitled “**STUDIES ON ENTOMOPATHOGENIC NEMATODES**” submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by him for a degree of any University.

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Date :

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## Certificate

This is to certify that the thesis entitled “**STUDIES ON ENTOMOPATHOGENIC NEMATODES**” submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr. SREERAM NANDA KRISHNA** under our guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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## **LIST OF ABBREVIATIONS AND SYMBOLS**

%	:	Per cent
@	:	At the relate of
°C	:	Degree Celsius
cm	:	centimeter
EPNs	:	Entomopathogenic nematodes
Fig.	:	Figure
gm	:	gram
hrs	:	Hours
i.e.	:	That is
IJS	:	Infective juveniles
LD	:	Lethal dose
LT	:	Lethal time
mg	:	milligram
min.	:	minutes
ml	:	milli litre
mm	:	millimeter
nm	:	Nanometer
U.V.	:	Ultraviolet
<i>viz.</i> ,	:	Namely

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### **ABSTRACT**

Studies related to survey for isolation of entomopathogenic nematodes, identification and mass multiplication, effect of environmental factors on nematode survival and pathogenicity and evaluation of entomopathogenic nematodes against three major lepidopteran pests were carried out in insectary, S.V. Agricultural College, Tirupati during November, 2004 to June, 2005.

Regarding isolation of entomopathogenic nematodes, no nematodes could be recorded from any of the total 135 samples collected. With regard to mass multiplication of entomopathogenic nematodes *G. mellonella* yielded highest progeny of *S. carpocapsae* of 2.30 lakhs per larva when 60 infective juveniles were inoculated. In the case of *H. indica*, there was more progeny production i.e. 4.34 lakhs with 80 infective juveniles. Both the nematodes yielded lowest progeny of 1.49 lakh juveniles of *S. carpocapsae* and 3.52 lakh juveniles of *H. indica* at 40 infective juveniles level. Large sized larvae (body size of 20-22 mm and weight of 340-350 mg) of final instar larvae of *G. mellonella* yielded maximum progeny of 1,02,400 juveniles of *S. carpocapsae* and 2,01,560 juveniles of *H. indica* whereas small sized larva (size of 10-12 mm and weight of 150 cm) yielded lowest progeny of 81,340 juveniles of *S. carpocapsae* and 1,06,000 juveniles of *H. indica*. Among the three host insects tested *G. mellonella* yielded highest progeny of both *S. carpocapsae* and *H. indica* i.e. 2.34 lakh juveniles and 4.1 lakh juveniles respectively followed by *C. cephalonica* 1.83 lakh juveniles of *S. carpocapsae* and 2.52 lakh juveniles of *H. indica* and *B. mori* 1.13 lakh juveniles of *S. carpocapsae* and 2.13 lakh juveniles of *H. indica*.

Temperature of 25°C was the optimum, recording 95.82 per cent survival of the infective juveniles of *S. carpocapsae*. However for *H. indica*, favourable temperature was 20°C recording maximum of 91.66 per cent survival of infective

juveniles. Highest temperature of 35°C was found to affect the survival of nematodes. Temperature showed its impact on pathogenicity against *G. mellonella* where 25°C was found to be optimum which resulted in 100 per cent mortality of the larvae by both nematode species. 35°C was proved to be most unfavourable for causing infection by *S. carpocapsae* and *H. indica* which has resulted the lowest mortality of 31.05 and 30.03 per cent respectively.

Influence of relative humidity on the survival of the infective juveniles revealed that 80 per cent relative humidity resulted in maximum survival of 79.72 per cent of *S. carpocapsae* and 79.50 per cent of *H. indica*. Whereas 50 per cent relative humidity resulted in lowest survival of 4.17 per cent of the infective juveniles of *S. carpocapsae* and 7.0 per cent of *H. indica*. Studies on effect of relative humidity on nematode pathogenicity revealed that highest relative humidity of 80 per cent showed 78.10 per cent by *S. carpocapsae* and 81.58 per cent by *H. indica*. At lowest relative humidity of 50 per cent, there was only 41.69 per cent larval mortality by *S. carpocapsae* and 50.00 per cent by *H. indica*.

Experiment on effect of U.V. radiation on survival of the entomopathogenic nematodes revealed that when the juveniles were exposed to U.V. radiation (254 nm) for a longer period of 120 minutes, it resulted in complete mortality of juveniles of both *S. carpocapsae* and *H. indica*.

Influence of pH on nematode survival revealed that pH of 7, maximum survival of 48.55 per cent *S. carpocapsae* and 55.12 per cent of *H. indica* was recorded. It also influenced the nematode pathogenicity were neutral pH of 7 recorded high larval mortality of 78.6 per cent by *S. carpocapsae* and 82.06 per cent by *H. indica*. 15 per cent soil moisture was suitable for both *S. carpocapsae* and *H. indica* that could show maximum survival of 78.62 and 80.41 per cent respectively. Whereas pathogenicity of both entomopathogenic nematode species recorded larval mortality of 75.97 per cent by *S. carpocapsae* and 79.99 per cent by *H. indica* at 15 per cent moisture level, which was recorded as optimum level.

Impact of soil type on nematode pathogenicity revealed that among three types of soils, black soil was more suitable for nematode activity as well as for causing mortality of the larvae of *G. mellonella*, which was recorded as 72.60 per cent by *S. carpocapsae* and 77.28 per cent by *H. indica*. Lowest of 58.20 per cent mortality by *S. carpocapsae* and 64.06 per cent by *H. indica* was recorded in sandy soil. Mortality was higher at 5 cm depth than 10 cm and 15 cm depth of soil.

Studies made on efficacy of the two entomopathogenic nematodes i.e. *S. carpocapsae* and *H. indica* against the control of three lepidopteran pests i.e. *H. armigera*, *S. litura* and *P. demoleus* revealed that among the three pests *P. demoleus* was more susceptible recording a maximum mortality of 75.73 per cent by *S. carpocapsae* and 70.45 per cent by *H. indica* followed by *H. armigera* showing 66.65 per cent by *S. carpocapsae* and 67.95 per cent *H. indica*. *S. litura* recorded a maximum mortality of 54.93 per cent by *S. carpocapsae* and 56.95 per cent *H. indica*. With increasing time period and level of inoculum, the values of LD<sub>50</sub> and LT<sub>50</sub> were gradually decreased.

## **CHAPTER - I**

### **INTRODUCTION**

Insect pests form an important limiting factor for crop production. Nearly one third of crop produce is estimated to be lost due to insect pests and mites. Sole reliance on pesticides to control the pests is not a sustainable approach because of the associated problems like environmental degradation, development of insect resistance to insecticides, pest resurgence and out break of secondary pests due to elimination of natural enemies etc. Due to these environmental hazards, it is clear that other effective, ecofriendly methods have to be incorporated in pest management practices. Biological control of pests is an ideal alternative, which is economical and can provide long-term control reducing risk to human beings, cattle and other non-target organisms and development of resistance and other hazards. This realization has contributed for a high annual growth in the sale of microbial based insecticides *viz.*, bacteria, fungi, nematodes, protozoa, viruses etc.

Nematodes associated with insects, referred to as entomophilic, entomogenous or entomopathogenic are known to parasitise, and kill the insects. The entomopathogenic nematodes (EPNs) serve as vectors of bacteria, which achieve quick kill of the target insect pests and thus have high potential in pest management.

During the last one decade, there has been heightened interest in augmentative biological control of insects using EPNs belonging to the families *Steinernematidae* and *Heterorhabditidae*. The infective juveniles of these nematodes carry the symbiotic bacterium in their intestine (*Xenorhabdus* in *Steinernematidae* and *Photorhabdus* in *Heterorhabditidae*) and release the bacterial cells after entering the insect haemocoel by way of natural openings (Spiracles, Mouth and Anus) and also directly through the soft cuticle of certain insects as in *Heterorhabditis*. The bacteria proliferate, kill the insect host, usually within 24 - 72 hrs and render it favourable for nematode development. In case of *Heterorhabditis*, infective juveniles develop into hermaphrodite females, and those of *Steineraema* develop into male and female progeny.

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* can be multiplied easily on final instar larvae of greater wax moth, *Galleria mellonella*, and Rice flour moth *Corcyra cephalonica*. Despite their impressive attributes, sensitivity of infective juveniles to inactivation by extremes of physical environments i.e. temperature, relative humidity, pH, moisture level and UV radiation prevent them from reaching their full potential.

The need for developing suitable nematode pesticides has gained urgency since many insect pests have become resistant to pesticides. The utilization of EPNs varies with soil born pests like *Spodoptera litura*, *Helicoverpa armigera*, *Papilio demoleus* which are capable of causing defoliation and greater economic loss to the crop. Entomopathogenic nematodes have the potential as bio-control agents against these pests which have their pupation in the soil, due to which, there is greater chance of attack by these entomopathogenic nematodes. Till now, the information is scanty on these bio-agents in Andhra Pradesh especially in Southern parts of the state. Hence, there is a need to buildup basic

information on entomopathogenic nematodes under the existing environmental conditions. Keeping this in view the present research work was planned under laboratory and insectary conditions in Department of Entomology, S.V. Agricultural College, Tirupati.

The programme was formulated with the following objectives:

1. Survey for isolation of local entomopathogenic nematodes.
2. Identification and mass multiplication of entomopathogenic nematodes.
3. Effect of environmental factors on entomopathogenic nematodes.
4. Evaluation of entomopathogenic nematodes for the management of important lepidopteran pests.

## **CHAPTER – II**

### **REVIEW OF LITERATURE**

Nematodes associated with insects are known as entomopathogenic or entomophilic nematodes (EPNs) which belong to two families, *Steinernematidae* and *Heterorhabditidae*. These are soil dwelling lethal parasites of insects that could be used for inundative, augmentative or inoculative biological control against crop pests. The infective juveniles of these EPNs are motile, virulent with high reproductive potential and possess the ability to seek out their hosts and potential for long term establishment in soil through recycling on infecting insects. A comprehensive review on isolation, mass multiplication, effect of environmental factors on entomopathogenic nematodes and evaluation of EPNs for the management of important lepidopteran pests by these bioagents is presented under following headings.

## **2.1 SURVEY FOR ISOLATION OF LOCAL ENTOMOPATHOGENIC NEMATODES**

### **2.1.1 Taxonomy of entomopathogenic nematodes**

Although 40 nematode genera associated with insects, only members of the two families *Steinernematidae* and *Heterorhabditidae* have been widely used as biocontrol agents. First entomo-pathogenic nematode was described as *Aplectana kraussei* by Steiner (1923) from Germany. Later Travassos (1927) erected the genus, *Steinernema* for this species. In 1929, Steiner proposed a new genus, *Neoplectana* and described as *Neoplectana glasseri*, but did not give clear differences between *Neoplectana* and *Steinernema*. Poinar (1976), has proposed the family Heterorhabditidae and the genus *Heterorhabditis* with the type species *H. bacteriophora* isolated from *Heliothis punctifera* from South Australia. Simultaneously, Khan *et al.*, (1976) proposed a new genus, *Chromonema* with its type species *Chromonema heliothidis* from *Heliothis zea* Poinar *et al.* (1977) proposed *Chromonema* as a synonym of *Heterorhabditis* and then *Chromonema heliothidis* became *Heterorhabditis heliothidis*. Later six more species were described by several researchers under the genus *Heterorhabditis* as *H. indica* by Poinar *et al.*, 1992;

*H. argentinensis* by Stock (1993); *H. brevicaudis* by Liu (1994);  
*H. hawailensis* by Gardner *et al.* (1994); *H. marellatus* by Lu and  
Berry (1966b) and *H. hepialius* by Stock *et al.* (1996).

The systematic position of *Steinernema* and *Heterorhabditis*  
is shown as:

Phylum	:	Nematoda
Class	:	Secernentea
Order	:	Rhabditida
Sub-order	:	Rhabditida
Super family:		Rhabditoidea
Family	:	Steinernematidae
Genus	:	<i>Steinernema</i>
Family	:	Heterorhabditidae
Genus	:	<i>Heterorhabditis</i>

### **2.1.2 Nematode – Bacterium Association**

The infective form of these entomopathogenic nematodes is a soil dwelling stage that can survive for several months without food. These carry one species of bacterium as symbiont within the lumen of its intestine. These nematodes while habitating in soil get attracted to an insect host (Bedding and Akhrust, 1975) and invade in to the host body through its natural openings. After entering into the digestive track of the insect, these penetrate into

the haemocoel of the host insect. In the haemocoel of insect host, the nematode then destroy the antibacterial protein (Gotz *et al.*, 1981) and releases its symbiotic bacteria. Nematodes of the families Steinernematidae and Heterorhabditidae are symbiotically associated with bacteria of the genus *Xenorhabdus* and *Photorhabdus* species respectively belonging to the family enterobacteriace (Akhurst, 1980). Bacteria found in *Steinernema* was found to be closely resembling *Xenorhabdus poinarii* while the bacterium associated with *H. indica* was identified as *Photothabdus* (Ambika, 1995). The bacteria proliferate within the haemocoel and kill the host by producing septicaemia within 48-72 hours. The bacteria can serve as a source of food material for the nematode development (Akhurst and Boemare, 1990, Poinar 1990).

**Table 1: Bacterial symbionts and associated nematodes.**

<b>Nematode</b>	<b>Symbiotic bacteriam</b>	<b>Reference</b>
<i>Steinernema carpocapsae</i>	<i>Xenorhabdus nematophilus</i>	Thomas, G.M and Poinar, G.O. 1979
<i>S. feltiae</i>	<i>X. bovienii</i>	Akhurst, R.J. 1980
<i>S. glaseri</i>	<i>X. poinarii</i>	Akhurst R.J., 1983, 1986
<i>S. intermedium</i>	<i>X. bovienii</i>	Poinar, 1985
<i>S. affinae</i>	<i>X. bovienii</i>	Boemar, N.E and Akhurst, R.J., 1988
<i>S. anomalae</i>	<i>X. bovienii</i>	Boemare <i>et al.</i> , 1993
<i>Heterorhabditis spp</i>	<i>Photorhabdus huminescens</i>	Boemare <i>et al.</i> , 1993

### **2.1.3 Isolation of entomopathogenic nematodes**

Soil was the natural habitat for entomopathogenic nematodes, where they were associated with different insects existing in crop ecosystems. They could be isolated from soil by baiting with susceptible host or from naturally infected insects. Simple and efficient baiting technique with greater wax moth *Galleria mellonella* was developed by Bedding and Akhrust (1975). Disease could be observed in about a week time in the baited trap insect (Hara *et al.*, 1991). These dead larvae were washed in distilled water and put on white trap for extraction of nematodes or dissected out to observe the presence of nematodes.

Gaugler (1981) conducted experiments for isolation of entomopathogenic nematodes and emphasized that infective juveniles of the entomopathogenic nematodes are very sensitive to environmental factors, such as temperature, relative humidity, soil moisture, pH and resulted that no nematodes were survived when atmosphere temperature was above 35°C.

Josephraj Kumar and Sivakumar (1997a) conducted a random survey for isolation of entomopathogenic nematodes in vilarancode kulkulm and Kanyakumari and reported prevalence of *Steinernema* species in 17 samples and *H. indicia* in one sample out of 163 samples. *Steinernema* was detected more in hilly regions with high rainfall and was more abundant in loamy soils.

Hussaini *et al.* (2001) conducted survey for isolation of entomopathogenic nematodes throughout the state and isolated various isolates of *S. carpocapsae* and *H. indica*, when the atmosphere temperature was below 25°C.

Prasad and Katti (2003) have collected soil samples from 20 sites and baited with *Corcyra cephalonica* for isolation of EPNs. Out of 20 samples larvae, in three samples showed the symptoms of infection by nematodes. The dead larvae were observed within 10 to 20 hours of baiting. Eswarmoorthy and Sankaranarayanan (2003) isolated *Heterorhabditis indica* from populations recovered from sugarcane top borer *Scirpophaga excerptalis* in the vicinity of Coimbatore.

## **2.2 IDENTIFICATION AND MASS MULTIPLICATION OF ENTOMOPATHOGENIC NEMATODES**

Efficacy of entomopathogenic nematodes as biocontrol agents depends on their amenability to mass production and formulation. Presently Steinernematidae and Heterorhabditidae nematode products are available world wide for control of insect pests, largely due to the progress made in mass production and which can be easily maintained on both *in vivo* and *in vitro* cultures.

### **2.2.1 Mass multiplication of entomopathogenic nematodes on *Galleria mellonella* by inoculating with different levels of infective juveniles**

The initial level of inoculum used for causing disease in the test insect also influence their final yield from the infected host.

According to PDBC (2002), the optimum level of IJS vary from 50 to 75 for causing infection and good progeny production in *G. mellonella* which is reported as high compared to other host like *Agrotis ipsilon* when used for mass multiplication. This is a common factor for both *Steinernema* and *Heterorhabditis*, in which *S. carpocapsae* yielded high progeny at 50 infective juveniles level, *H. indica* at 75 infective juveniles level.

### **2.2.2 Yield of entomopathogenic nematodes from different larvae of different sizes of greater wax moth *Galleria mellonella***

Raj Kumar *et al.* (2002) conducted experiment on mass multiplication of entomopathogenic nematodes from Udaipur using *Galleria mellonella* of three types larvae based on the body size and weight. Maximum number of *Steinernema species* 90,945 IJS per larva were recorded in large sized larva (body size of 20-22 mm). Similarly in case of *Heterorhabditis species*, maximum number of IJS 2,01,520 per larva could be in the large sized larva (body size of 23-25 mm).

### **2.2.3 Progeny production of entomopathogenic nematodes from different insect hosts**

Though *G. mellonella* was used as a host for mass multiplication of EPNs many number of insect hosts were also tested for their ability in producing large number of progeny. Where *Steinernema species* and *Heterorhabditis species* were evaluated in different lepidopteran pests.

Karunakar *et al.* (1992) reared *Steinernema feltiae*, *Steinernema glaseri* and *Heterohabditis bacteriophora* on *Chilosacchariphagus indicus* and *Scirpophaga excerptalis* where he could able to harvest 1,27,000 IJS of *S. feltiae*; 3,70,000 IJS of *S. glaseri* and 2,10,000 IJS of *H. indica* from fifth instar larvae of *Chilo sacchariphagus indicus* when inoculated @ 20 IJS per larva.

Hussaini *et al.* (1998, 2001a) reported maximum yield of isolates was observed in final instar larvae of *G. mellonella* of all the species (2.18 lakhs). *H. bacteriophora* recorded the highest yield of 5.4 lakhs in *G. mellonella* followed by *Agrotis ipsilon*, *Corcyra cephalonica*, *Spodoptera litura*, *Helicoverpa armigera*.

As per the Reports of Project Directorate of Biological Control P.D.B.C. (2001). *Spodoptera litura* could produce 3.56 lakh juveniles of *H. bacteriophora* per larva and *Heliothis armigera* 3.16 lakh juveniles of *H. bacteriophora* per larva. Among *H. bacteriophora* species tested progeny production of *H. bacteriophora* 13.3 was higher in *H. armigera*, *Opisina arenosella*, *Plutella xylostella* when tested for their production.

Zaki *et al.* (2000) used silk worm larvae *Bombyx mori* for *in vivo* culturing of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, where he could able to harvest an average of 2750 and 48,703 juveniles of *H. bacteriophora* and *S. carpocapsae* respectively from third instar larvae. However, no nematodes could be recovered from the fifth instar larvae of silk worm.

Parimala Gupta (2003) has done *in vivo* culturing of *Steinernema carpocapsae* by placing fourth instar larvae of *Corcyra cephalonica* on Whatman 1 filter paper by inoculating with *S. carpocapsae* @ 20 IJS per larva and the inoculated petridishes were incubated at 20 – 24°C. After 10-12 days IJS emerged were harvested from the cadavers.

### **2.3 EFFECT OF ENVIRONMENTAL FACTORS ON ENTOMO-PATHOGENIC NEMATODES**

Among different ecological factors temperature, moisture, relative humidity, U.V. radiation, pH and soil texture play an important role in the survival and infectivity of entomopathogenic nematodes. These further influence the time for disease development and multiplication.

### **2.3.1 Effect of temperature on nematode survival**

Soil temperature had a direct effect on survival and infection of Heterorhabditid and Steinernematid nematodes.

Though Steinernematids are active over a broad range of temperatures (Danilov, 1976), various studies showed that air temperature of 25°C was optimum for growth and development. However, at high temperatures at 32°C can cause quicker mortality of *G. mellonella* and normal mortality could be observed at temperatures ranges from 10-32°C when infected with *S. carpocapsae*.

The optimum temperature for good development of *S. carpocapsae* reported was 25°C by Pye and Burman (1978).

Molyneux (1986) reported that acceptable temperature for nematode survival, development and infection for disease development vary with nematode species and strain. *S. carpocapsae* was found to be most effective at all

temperatures ranging from 10 to 28°C as compared to *S. feltiae* and *H. heliothidis*..

Westerman *et al.* (1989) reported that at 8 - 10°C under field conditions an isolate of *Heterorhabditis species* (Hf 85) could cause 100 per cent mortality of the curculionid *Otioryncus sulcatus*.

Mason and Hominick (1990) reported that the survival of a British isolate of *Heterorhabditis species* was greatest at 7°C and lowest at 15°C in the absence of hosts.

Survival and pathogenicity of *S. carpocapsae* was significantly greater at lower temperatures i.e. 5 to 25°C than at the highest temperatures of 35°C (Shang Ping Kung *et al.*, 1991).

Ghally (1995) reported that an optimal soil temperature of 20-25°C was suitable for the activity of *Heterorhabditis heliothidis* and *Steinernema carpocapsae* and to infect the cotton leaf worm *Spodoptera littoralis*.

Grewal *et al.* (1996) reported that the thermal reproductive niche of *H. bacteriophora* was extended from 15-30°C to 12-32°C.

Maximum survival of *H. indica* was achieved at 15°C and highest mortality was observed at 5-25°C (Strouch and Ehlers, 2000).

### **2.3.1.1 Effect of temperature on pathogenicity of entomopathogenic nematodes**

Gaugler and Mulloy (1981) conducted studies on the effect of temperature on the infectivity and host mortality, which indicated that even at a low temperature of 9°C, *S. carpocapsae* could cause mortality of the host, but infection takes longer period of 312 hours (14-15 days). Where host mortality occurred within 16 hours when the temperature was maintained as 30°C.

Zervos *et al.* (1991) conducted laboratory studies with *G.mellonella* by inoculating with different inoculum levels of IJS of 5, 10, 25, 50, 100 and 500 IJS per larva of *H. heliothidis* and *S. glaseri* at different temperatures of 5, 10, 15, 20, 25 and 30°C and concluded that no emergence of juvenile was observed at temperature 5 or 10°C. Development was most rapid at 25°C. No

emergence of the *H. heliothidis* was observed at 30°C when inoculated with 500 IJS per host. However, emergence of IJS was observed early at high temperatures, where the host was inoculated with large number of IJS. Yield per host was greatest at 20°C even with low level of IJS inoculation.

Gouge *et al.* (1994) assessed the infection of *G. mellonella* by *S. feltiae* and *S. carpocapsae* between 6.4 and 19.2°C after storage of IJS in sterile sand. Both nematodes infected the host at temperature as low as 11 and 13.79°C respectively. However *S. carpocapsae* caused no mortality of the host below 15.92°C. The infectivity of *S. carpocapsae* at 20°C was significantly higher when the nematode had been stored at low temperature 6.4°C.

Schirocki and Hague (1994a) studied the temperature profiles for two isolates of *S. carpocapsae* (All and UK) and two isolates of *Heterohabditis, nemasys - H* and fight grub against late instar larvae of *Otiorhynchus sulcatus*. Efficacy was clearly delineated for each isolate of both nematodes based on the temperature evidence as 14.33°C for *S. carpocapsae*, 14-28°C for *Namasys-H* and 14-33°C for fight grub.

Williams and Macdonald (1995) reported that *S. feltiae* and *Heterohabditis species* (Strain UK 211) caused a significant reduction in the number of dipteran leaf miner larvae *viz.*, *Liriomyza huidobrensis* at a temperature range at 10-30°C.

Sham Seldean *et al.* (1996) studied the effect of soil temperature on efficacy of four species of entomopathogenic nematodes in infecting and killing *Spodoptera littoralis* in the laboratory in relation to soil temperature, nematode dose and emergence from the insect cadavers. Results revealed that all the tested *H. bacteriophora* nematodes caused 100 per cent mortality of *S. littoralis* at 4, 10 and 25°C *H. bacteriophora* (HP 88) achieved the least mortality at 35°C. All nematodes could infect and kill the host insects at 35°C.

Karunakar *et al.* (1999) studied the influence of eight temperature levels *viz.*, 10, 12.5, 15, 25, 27.5, 30, 32.5 and 35°C on infection, penetration and multiplication of *S. feltiae*, *S. glaseri* and *H. indicus* using larvae of *G. mellonella*. The nematode infection and penetration in *G. mellonella* was observed from 12.5

to 32.5°C, multiplication was observed from 25.0 to 32.5°C. No host mortality was observed at 10 and 35°C Steinernematids showed significantly higher penetration into the host at 25°C. While more by *H.indicus* at 27.5°C. Multiplication was significantly higher in *H. indicus* followed by *S. feltiae* and *S. glaseri*.

Lacey and Unruh (1998) studied the effect of temperature on the susceptibility of larvae of the codling moth, *cydia pomonella* to *S. carpocapsae*, *S. riobrave* and *H. bacteriophora* at temperature levels of 15, 20, 25 and 30°C. Concluded that *S. carpocapsae* was the most effective in causing the highest host mortality ranging from 66 to 90 per cent at 25°C than other nematodes.

### **2.3.2 Effect of relative humidity on survival of entomopathogenic nematodes**

Relative humidity is the important environmental factor that influence the entomopathogenic nematodes survival and disease causing ability in the host insect.

Simons and Poinar (1973) demonstrated that *Steinernema carpocapsae* survived in relative humidities well below the permanent wilting point of plants. Longest survival of nematodes i.e. 80 per cent survival of the nematodes was observed at 85 per cent humidity level over a period of 102 hrs.

Kamionek *et al.* (1974) conducted experiment on the effect of relative humidity on survival of the nematode infective juveniles and reported that at 85% humidity level 98% survival of *S.carpocapsae* was observed.

Shang Ping Kung *et al.* (1991) conducted experiment to test the survival of the two nematode species *S.carpocapsae* and *S.glaseri* under laboratory conditions, reported that survival of these two nematode species decreased as RH decreased from 100 to 25%.

#### **2.3.2.1 Effect of Relative Humidity on pathogenicity of entomopathogenic nematodes**

Moore (1965) reported that *S.carpocapsae* survived for 20 days in moist soil when it was slowly dried at 70% humidity level,

and pathogenicity will be increased with increasing level of humidity.

Shang Ping Kung *et al.* (1991) tested the effect of relative humidity on the pathogenicity of *S.carpocapsae*, *S.glaseri* on *G.mellonella* and reported that pathogenicity of these two nematode species decreased as RH decreased from 100 to 25%.

### **2.3.3 Effect of UV radiation on survival of entomopathogenic nematodes**

Ultraviolet portion of solar radiation is generally regarded the most destructive abiotic factor affecting the persistence of entomopathogenic nematodes.

Gaugler and Boush (1978) reported loss of pathogenicity in *Steinernema feltiae* when IJS were exposed to UV radiation (240 nm) for seven minutes. When further exposed for 2.5 to 5.0 minutes the reproduction and development were also inhibited.

Randy Gaugler *et al.* (1992) conducted experiment to test effect of UV radiation on nematode survival and results revealed

that complete inactivation of *H.bacteriophora* resulted following 4 min of irradiation, compared with 10 min for *S.carpocapsae*.

Nickel and Shapiro (1994) found that Blankophor and Tinopal were the most successful UV screens, for *S. carpocapsae* which retain 95 per cent infectivity of the larvae of *G. melloaella* even after four hours of exposure to sun light. Similarly they recorded 80 and 85 per cent infectivity preserved by Blankophor HRS and DML and P 167 preserved, 70 per cent infectivity after the 4 hours sun light exposure.

Cheng Chichin (1997) reported that all nematodes i.e. *Steinernematis* and *Heterorhabditis* species were dead after continuous exposure of nematodes to 254 nm UV light for 36 hours.

Ragaei (1999) suggested flour encapsulation of nematodes in addition to antioxidants and oxidative enzymes for the protection of nematodes from UV radiation.

Gulsar Banu and Rajendran. (2003) conducted experiment to test the effect of UV radiation on nematode survival and revealed that they have observed the absolute mortality of the injective juveniles of *S. glaseri*, *H. indica* with continuous exposure to UV radiation for 120 -135 min.

#### **2.3.3.1 Effect of UV radiation on pathogenicity of entomopathogenic nematodes**

Randy Gaugler *et al.* (1992) conducted experiment to test the effect of UV radiation on nematode pathogenicity against *G.mellonella* and revealed that loss of pathogenicity in *H.bacteriophore* occurred at 4 min irradiation whereas 6 min for *S.carpocapsae*.

Regarding pathogenicity of *H.indica* and *S.glaseri*, Gulsar Banu and Rajendran (2003) reported that the percent infectivity of irradiated juveniles on *G.mellonella* decreased with increase in exposure period.

#### **2.3.4 Effect of pH on survival of entomopathogenic nematodes**

Fischer and Fuhver (1990) reported that acidic soil with pH levels below four limited the movement of *S. kraussei* in finding the host.

Kung *et al.* (1990b) reported that survival of *S. carpocapsae* and *S. glaseri* gradually declined during 16 weeks observations as the tested pH decreased from eight to four, survival of both species was dropped sharply after one week at a pH 10. Survival of both species was similar at pH 4, 6 and 8 during first four weeks. However, the capacities of species vary according to the pH levels. Where *S. carpocapsae* survival was significantly greater than *S. glaseri* at pH 10 for 16 weeks.

Cheng Chichin (1997) reported that when the IJS incubated in phosphate buffer of pH of 4-12 for 10 days, the IJS could maintain about 60 to 70 per cent survival rate. When the same pH of the suspension was adjusted to two, all the IJS were found dead.

#### **2.3.4.1 Effect of pH on pathogenicity of entomopathogenic nematodes**

Kung *et al.* (1990b) conducted experiment to test the effect of pH on nematode pathogenicity against *G.mellonella* and reported that *S. carpocapsae* and *S. glaseri* that were stored at pH 4, 6 and 8 for 16 weeks and at pH 10 for one or more weeks could infect the larvae of *G. mellonella*.

#### **2.3.5 Effect of moisture on survival of entomopathogenic nematodes**

The nematode infectivity was found to be decreased at low water potential (>-10 bars) in fine sand or clay loam soil or at very high water potential (<0.01 bars) in clay loam soil (Molyneux and Bedding, 1984).

Water saturated soil was found to be detrimental for survival of entomopathogenic nematodes as the oxygen in soil was low beside impairing their active movement (Kaya, 1990). *S. carpocapsae* and *S. glaseri* survival best at low moisture content of two and four per cent respectively (Kung *et al.*, 1990a).

Shang Ping Kung *et al.* (1991) tested the effect of soil moisture on survival of the infective juveniles of the two nematode species i.e. *S.carpocapsae* and *S.glaseri* and reported that both nematode species survived best at low moistures of 2 and 4% respectively.

Wang Qinglei *et al.* (1995) reported that *S. glaseri* moved horizontally a little more than 40 cm in 48 hours, when the water content of soil has kept at least 19 per cent with the reduction of soil, water content nematodes mobility was affected.

#### **2.3.5.1 Effect of moisture on pathogenicity of entomopathogenic nematodes**

Ghally (1995) reported that the activity and pathogenicity of *H. heliothidis* and *S. carpocapsae* to the cotton leaf worm *Spodeptera littoralis* increase as the soil moisture was increased to field capacity level.

Shang Ping Kung *et al.* (1991) reported that rehydration by bringing up all tested moistures to 16%, both nematode species

i.e. *S.carpocapsae* and *S.glaseri* caused lethal infection of the larvae of *G.mellonella*.

### **2.3.6 Effect of soil type on pathogenicity of entomopathogenic nematodes**

More than 90 per cent of the insects spend part of their life cycle in the soil and consequently use of nematodes in this habitat is very promising. So that nematode pathogenicity vary in different soils.

The infectivity of *S. glaseri* and *Heterorhabditis species* was found to be less in clay loam soil with high clay content than clay soil (Molyneux and Bedding, 1984 and Geden *et al.*, 1985).

Soils enriched with humus with high organic matter harboured more nematodes (Hominick and Briscoe, 1990).

Kung *et al.* (1990a) reported that the survival of *S. carpocapsae* and *S. glaseri* was lowest in clay soil in comparison to clay loam and sandy loam. *S. carpocapsae* was most active in sandy loam and *S. glaseri* was most active in sandy soil.

Occurrence of *Heterorhabditis* was highly correlated with ocean beaches containing a sand grains from coral and shell with pH of 8 and low organic matter content. Whereas Steinernematid isolates were found in silty clay and silty loam soils with high organic matter content (Hara *et al.*, 1991).

High frequency of nematodes i.e. *Heterorhabditis species*, *S. affinae* and *S. feltiae* from sandy and peaty soils than clay and clay loam soils was reported by Giffin *et al.* (1991).

Barbercheck and Kaya (1991a) conducted laboratory experiment to determine the effect of soil texture and condition of larvae of *G. mellonella* on host finding by *H. bacteriophora* and *S. carpocapsae*. The results revealed that *H. bacteriophora* was more motile than *S. carpocapsae* in organic and fine sandy loam soils and less in clay soil. Hsiao – Wenfeng *et al.* (1996) reported that *S. carpocapsae* moved more readily in sand and silty loam than in coarse sandy loam or sandy clay loam soils. When IJS were placed on the soil surface, they displayed little down ward movement was observed at temperatures of 20, 25 and 35°C in all

soil types. At 35°C, no nematodes were recovered from 6 cm (deeper) however, they only tended to move to the next 5 cm layer. Higher numbers of nematodes were recovered from silty loam when placed at a 5 cm depth, and higher numbers were recovered from sandy clay loam when placed at 10 cm depth. Indicate that the migration of IJS tends to decrease as the proportions of silt and clay increase in soil.

Hussaini *et al.* (2000) reported that performance of four native species of nematodes were found to be better in sandy loam soil than in sandy soil at 5 cm depth against *Agrotis ipsilon*. Combination of *S. carpocapsae* PDBC En 6.11 and *H. indica* PDBC EN 13.22 had an additive effect over their individual populations in both soil types at 10 cm depth.

Hussaini and Sankaranarayanan (2001) recorded absolute mortality of *G. mellonella* in which sandy and sandy loam soils injected with *Steinernema species* and *Heterorhabditis* at 5 and 10 cm depth. However, infectivity of *Steinernema species* was higher at 5 cm depth and progeny was also higher from kept in sandy loam soils.

## **2.4 EVALUATION OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF IMPORTANT LEPIDOPTERAN PESTS**

Entomopathogenic nematodes have tremendous potential as biocontrol agents against Lepidoptera, Coleoptera and Orthoptera pests. Among all, these EPNs are effective against Lepidopteran pests which pupates in the soil and gets the chance of nematode infection in the soil.

### **2.4.1 Pathogenicity of *S. carpocapsae* / *H. indica* on *Helicoverpa armigera***

Pathogenicity of entomopathogenic nematodes from the genera, *Steinernema* and *Heterorhabditis* was tested by Glazer and Navon (1990). They recorded complete mortality of *H. armigera* inoculated with 200 IJS of *S. feltiae filipjev*, strain all and found that 54 IJS per insect was the LD<sub>50</sub> and period of eight hours of insect exposure to the nematode was required to induce more than 80 per cent mortality.

Cabanillas and Rawston (1992) tested the efficacy of *Steinernema riobravis* in suppressing the larvae and pre pupae of *Helicoverpa zea* where the IJS @ 2 billion nematodes per ha were applied to soil and observed 95 – 100 per cent disease in both larvae and pupae.

Tohirand Otto (1995) investigated the effect of *Steinernema riobravis*, *Steinernema carpocapsae* and *Heterorhabditis species* on *Helicoverpa armigera*. He reported that there was linear relationship between the dosage of nematodes and the number of nematodes established. *H. armigera* was more susceptible to *S. riobravis* than others.

#### **2.4.2 Pathogenicity of *S. carpocapsae* / *H. indica* on *Spodoptera litura***

When two species of entomopathogenic nematodes, *Steinernema carpocapsae*, *S. feltiae* were applied as a foliar application for the control of *Spodoptera littoralis*, *S. carpocapsae* was recorded as less effective than *S. feltiae* (Williams and Walters, 1999).

Navon and Nagalakshmi (2002) observed cent per cent mortality of fourth instar larvae of *Spodoptera littoralis* and *Helicoverpa armigera* by feeding on 1000 IJS per gm of *S. carpocapsae* in the form of gel for 24 hours. Where as 70 - 100 per cent mortality was observed when 500 IJS / gm of *S. carpocapsae*. was infected to second to fifth instars of *H. armigera* and third, fourth, fifth and sixth instars of *S. littoralis*.

Prabhuraj *et al.* (2002) observed pathogenicity of *Steinernema glaseri* and *Heterorhabditis minutus* on final instar larvae of *Spodoptera litura* and concluded that LC<sub>50</sub> value for *S. glaseri* was 1165.2 and *H. minutus* was 424168 IJS per larva.

Hussaini *et al.* (2003) reported that among the different dosages of EPNs tested i.e. 100 IJS of *Heterorhabditis* and 125 IJS of *Steinernema species* were effective against *Spodotera litura* among different isolates i.e. *S. tami* 2.1, *S. abbasi* 3.1, *S. biocornutum* 3.2, *S. carpocapsae* 6.11, 6.61, 13.1 and *H. indica* 13.3. The pre cent invasion into the larvae of *S. litura* was highest in *S. abbasi* 3.1 followed by *S. carpocapsae* and *S. bicornutum*.

Environmentally sound control of *Spodoptera depravata* with Korean entomopathogenic nematodes *Heterorhabditis* species Gyeongsan, *S. carpocapsae* GSN, *S. glaseri* Dongrae, *S. longicaudum* Nonsane, *S. monticolum* was evaluated under laboratory conditions by Kang younglin *et al.*(2004). They observed corrected mortality of 53.3 – 66.7 per cent of 3<sup>rd</sup> instar larvae of *Spodoptera depravata* larvae after 5 days of inoculation. The LC<sub>50</sub> values of *S. carpocapsae* GSN and *S. monticolum* were reported as 6.9 and 3.9 respectively against 3<sup>rd</sup> instar larvae of *S. depravata*.

#### **2.4.3 Pathogenicity of *S. carpocapsae*/ *H. indica* on *Papilio demoleus***

Singh (1993) studied cross infectivity of EPNs where the *Steinernema* species isolated from *Agrotis segetum*, were tested against second instar caterpillars of *Papilio demoleus* and he could able to observe mortality of (45%) *P. demoleus* within 48 hours. Further, mortality of 10 to 50 per cent of the surviving caterpillars was observed after another five days. Cumulative mortality of 90 - 100 per cent could be recorded with in 10 days after inoculation.

**Table 2: List of Entomopathogenic nematodes used in the management of different crop pests.**

<b>Pest</b>	<b>Nematode</b>	<b>Reference</b>
<i>Scirpophaga incertulas</i>	<i>Steinernema carpocapsal</i>	Rao and Manjunath 1966
	<i>Heterorhabditis indica</i>	Israel <i>et al.</i> , (1969 a)
		Yadava and Rao 1970
<i>Spodoptera litura</i>	<i>S. carpocapsae</i>	Singh and Bardhan 1974
	<i>S. carpocapsae</i>	Rajeswari sundarababu <i>et al.</i> , 1984
	<i>S. carpocapsae</i> and <i>S. feltiae</i>	Narayanan and Gopalkrishnana 1987
<i>S. litura</i> , <i>H. armigera</i>	<i>S. carpocapsae</i>	Gupta <i>et al.</i> , 1987
<i>Helicoverpa armigera</i>	<i>S. carpocapsae</i> and <i>S. feltia</i>	Ghode <i>et al.</i> , 1988
<i>Coryra cophalenica</i>	<i>H. bacteriophora</i>	Sivakumar <i>et al.</i> , 1989
<i>Spodoptera litura</i>	<i>H. bacteriophora</i>	Sivakumar <i>et al.</i> , 1989
<i>Cylas formicarius</i>	<i>S. carpocapsae</i>	Jannsen <i>et al.</i> , 1990
<i>Spodoptera litura</i>	<i>S. feltiae</i>	Singh <i>et al.</i> , 1992
<i>Spodoptera littoralis</i>	<i>S. carpocapsae</i>	Glazer <i>et al.</i> , 1992
<i>Holotrichia censanguinea</i>	<i>S. carpocapsae</i> DD136 <i>S. glaseriou</i> (34)	Shanthi and Sivakumar, 1991
<i>Cnaphalocrocis medinalis</i>	<i>S. carpocapsae</i>	Srinivas and Prasad 1991
<i>Scirpophaga excerptalis</i>	<i>H. Indica</i>	Poinar <i>et al.</i> , 1992
<i>Spodoptera litura</i>	<i>S. glaseri</i>	Vyas and Yadav <i>et al.</i> , 1993
<i>Papilio spp.</i>	<i>Steinernema spp.</i>	Singh 1993
<i>Amsacta albistriga</i>	<i>S. carpocapsae</i>	
	<i>H. indica</i>	Bhaskaran <i>et al.</i> , 1994
	<i>H. bacteriophora</i>	
<i>Pieris brassicae</i>	<i>S. feltiae</i>	Mathur <i>et al.</i> , 1994
	<i>S. carpocapsae</i>	
<i>Phyllocnistis citrella</i>	<i>S. carpocapsae</i>	Beatie <i>et al.</i> , 1995
<i>Listronotus oregonensis</i>	<i>S. carpocapsae</i>	Belair and Boivin 1995
<i>Spodoptera litura</i>	<i>S. carpocapsae</i>	Sezhian <i>et al.</i> , 1996
<i>Cydia pomonella</i>	<i>S. carpocapsae</i>	Laciy and Unruh 1998
<i>Phitella xylostella</i>	<i>S. carpocapsae</i>	Yang <i>et al.</i> , 1999
	<i>H. bacteriophora</i>	

<b>Pest</b>	<b>Nematode</b>	<b>Reference</b>
<i>H. armigera</i>	<i>Steinernematids</i>	Vyas and Yadav <i>et al.</i> , 1998
<i>Diapropes abbreviatus</i>	<i>S. carpocapsee</i>	Shapiro and Mc coy 2000
<i>Holotrichia serrata</i>	<i>H. Indica,</i>	Karunakar <i>et al.</i> , 2000
	<i>S. glaseri</i>	
<i>Agrotis ipsilan</i>	<i>S. carpocapsae</i>	Hussaini <i>et al.</i> , 2000
<i>Plutella xylostella</i>	<i>S. carpocapsal</i>	
	<i>S. feltiae,</i>	Shinde and Singh (2000)
	<i>Heterorhabditids</i>	
<i>Bombyx mori,</i>		
<i>C. cephaloaica</i>	<i>H. bacteriophore</i>	Zaki <i>et al.</i> , (2000)
<i>Leucinodes orbonalis</i>	<i>S. carpocapsae</i>	Hussaini <i>et al.</i> , 2002
	<i>H. indica</i>	
<i>Spodoptera litura</i>	<i>Heterorhabditis sp.</i>	Rajkumar <i>et al.</i> , 2002
<i>S. litura</i>	<i>H. indica</i>	Sitaramaiah <i>et al.</i> , 2003
<i>Agrotis ipsilon</i>	<i>S. carpocapsae</i>	
	<i>S. abbari</i>	Hussaini <i>et al.</i> , 2003
	<i>H. indica</i>	
<i>Heliothis armigera</i>	<i>H. indica</i>	PDBC (2001)

## **CHAPTER - III**

### **MATERIALS AND METHODS**

Insect parasitic *Rhabditid* nematodes of the genera *Steinernema carpocapsae* and *Heterorhabditis indica* possess tremendous potential as bio-control agents for a range of insect pests of agricultural importance. Keeping in view soil samples were collected in and around Tirupati region for isolation of entomopathogenic nematodes, experiments for mass multiplication, effect of environmental factors on nematode survival and nematode pathogenicity against lepidopteran pests were carried out in laboratory and insectary, Department of Entomology, S.V.Agricultural College, Tirupati. Materials used and methods followed for the above mentioned objectives are presented in this chapter.

### **3.1 SURVEY FOR ISOLATION OF LOCAL ENTOMOPATHOGENIC NEMATODES**

Insect parasitic nematodes can be recovered from naturally infested soils by baiting susceptible host insects (Bedding and Akhrust, 1975). Hence, keeping in view soil samples were collected for isolation of entomopathogenic nematodes.

#### **3.1.1 Collection of soil samples**

For isolation of entomopathogenic nematodes survey was carried out during January to June, 2005. For this 35 soil samples were collected in “Pacchikapulam” village of Vedurukuppam mandalam where soils of different cropping systems like rice, sorghum, maize, bajra, cotton, vegetable crops like tomato, brinjal, ladies-finger, ornamental crops like jasmine and rose, fruit crops like mango, citrus and guava. Twenty samples from Chandragiri region, 20 samples from Tirumala hills, 10 samples from Karakambody, 20 samples from RARS, Tirupati and 30 samples from Mahanandi region were collected during the course of study. Samples were collected from 10-15 cm layers of soil around the root zone of the crops by using hand hoe. From the

composite sample, 500 gms of soil was finally drawn for baiting. Samples were placed in plastic containers and each container was baited with two to three final instar *Galleria mellonella* larvae. Sample containers were covered tightly with lids containing small holes to facilitate gaseous exchange and were kept at room temperature ( $25 \pm 2^{\circ}\text{C}$ ). Larval mortality was recorded at 24 hours interval upto five days.

### **3.2 IDENTIFICATION AND MASS MULTIPLICATION OF ENTOMOPATHOGENIC NEMATODES**

Identification of the nematode infection was done based on the colour change of the test insect. In case of *S. carpocapsae* the infected cadavers turned to light brown colour (Plate 1) and *H. indica* infected cadavers turned to dark red colour (Plate 2).

Key factor in the success of EPNs as bio-pesticides is their amenability for mass production. Their mass production and subsequent formulation are the important issues in their commercialization. *In vivo* culture method was followed for mass production of both *S. carpocapsae* and *H. indica*.

### **3.2.1 Nematode culture**

Nematode cultures of both *S. carpocapsae* and *H. indica* were obtained in the form of capsules from project directorate of biological control (PDBC), Hebbal, Bangalore and used for further mass multiplication.

### **3.2.2 Maintenance of laboratory hosts**

#### ***3.2.2.1 Maintenance of greater wax moth (G. mellonella)***

Nematodes have been successfully cultured on greater wax moth *Galleria mellonella* (Woodring and Kaya, 1988). So that culture of greater wax moth were maintained on artificial diet under laboratory conditions. Artificial diet was prepared with the following ingredients

Wheat flour	:	100 gm
Corn flour	:	200 gm
Wheat bran	:	100 gm
Milk powder	:	100 gm
Yeast extract powder	:	50 gm
Honey	:	175 ml
Glycerin	:	175 ml

Corn flour, wheat flour, wheat bran, milk powder and yeast extract powder were mixed thoroughly in a bowl. Honey and glycerin were added in separate container which was added to the flour mixture by mixing thoroughly. Healthy larvae of *G. mellonella* were taken and released into diet in the rearing jars and allowed to continuation of their life cycle. Later pupae of *G. mellonella* were separated from the diet and were kept separately in plastic containers for adult emergence. The emerged adults were released into plastic containers for laying eggs on a white card board with folded paper strips to provide sites for egg laying (Plate 3). Cotton dipped in honey solution was kept as food for adults. After egg laying the papers with egg mass kept in artificial diet for emergence of the larvae. Later the diet was provided to larvae in sufficient quantities. Uniform aged larvae were used for mass production.

#### **3.2.2.2 Maintenance of rice moth (*Corcyra cephalonica*)**

Larvae of rice meal moth *Corcyra cephalonica* were collected from a godown in Agricultural Research Station and were reared on broken sorghum grains and groundnut seeds. Uniform aged larvae were used for mass production.

### **3.2.2.3 Maintenance of Silkworm (*Bombyx mori*) culture**

Before commencement of silkworm rearing all the rearing equipments were washed thoroughly with water, disinfected by spraying four per cent formaldehyde solution. Then the egg sheets obtained from near by rearing centre were spread out in a single layer in rearing trays and covered with paraffin paper. To ensure uniform hatching when the eggs attained blue stage (about 24 hours prior to hatching) egg sheets were put into wooden box and wrapped up in black sheet of paper. Next day they were exposed to bright day light. Two hours after hatching by adopting direct feeding method larvae were brushed on to wooden trays having paraffin paper as its floor. Upto third instar feed was provided by cutting into small pieces. After that feed was provided two times per a day. Fourth instar larvae were used for mass multiplication of entomopathogenic nematodes.

### **3.2.3. Mass multiplication of entomopathogenic nematodes on *Galleria mellonella* by inoculating with different levels of infective juveniles**

Both *S. carpocapsae* and *H. indica* were sub-cultured on larvae of *G. mellonella* as per the method described by Bedding

(1984). The healthy final instar larvae of *G. mellonella* were released into the glass rearing chamber prepared by keeping a moist filter paper over a petridish. Then the larvae were inoculated with infective juveniles at the rate of 20, 40, 60 and 80 per larvae. These petridishes were covered with parafilm to avoid evaporation of moisture as well as secondary infection by parasites. These were kept at room temperature  $25 \pm 2^{\circ}\text{C}$  for release of nematodes.

#### **3.2.4 Yield of entomopathogenic nematodes from larvae of different sizes of greater wax moth *Galleria mellonella***

For finding suitable size of the final instar larvae of *Galleria mellonella* larvae of different sizes were used, based on the size and weight. For this ten small sized (body length of 10-12mm and weight of 150mg), medium sized (body length of 14-16 mm and weight of 240 mg) and large sized (body length of 20-22 mm and weight of 350 mg) larvae were taken into separate petriplates over a filter paper. Then larvae were inoculated with infective juveniles of both *S. carpocapsae* and *H. indica* at the rate of 60 infective juveniles per larva. After that dead cadavers were transferred to white trap and kept for incubation at  $25 \pm 2^{\circ}\text{C}$ . After that the nematodes emerged were harvested.

### **3.2.5 Progeny production of entomopathogenic nematodes from different insect hosts**

Experiment was conducted to find out the suitable host insect for mass multiplication of *S. carpocapsae* and *H. indica* under laboratory conditions. For this final instar larvae of *G. mellonella*, *Corcyra cephalonica* and fourth instar larvae of *Bombyx mori* were used. Ten larvae of each test insect were taken into petridishes over a filter paper and inoculated with 1 ml nematode suspension (60 IJS/larva) of both *S. carpocapsae* and *H. indica* (Plate 4 to 7). Then the dead cadavers were transferred to white trap and kept for incubation at  $25 \pm 2^{\circ}\text{C}$ . Emerged nematode juveniles were harvested.

### **3.2.6 Harvesting of nematodes**

Four days after incubation the infected larvae were placed on white trap (White, 1927) (Plate 8 & 9), which consists a glass petriplate (9 x1.5cm) with watch glass. The infected cadavers were placed on the watch glass and the petridish was covered with parafilm and kept in incubator at room temperature  $25 \pm 2^{\circ}\text{C}$ . Since the first emergence, IJS were harvested with distilled water

in to a beaker upto four to five days. Harvested nematodes were washed with 0.1 per cent hymine solution, filtering through filter paper. In this manner nematodes were cleaned three to four times with distilled water. From the total nematode solution 50 $\mu$ l was pipetted with micropipet and counted the number of nematodes and calculated total nematodes, harvested. Freshly harvested nematodes were stored in a broad petriplates mixing with 2 per cent formaline to increase stability.

### **3.2.7 Preparation of entomopathogenic nematode juveniles into capsule formulation**

Poor storage and persistence in the field are the major obstacles in the expanded use of EPNs as bio-insecticides. Hence, formulation is very essential which is intended to improve activity, absorption, delivery and care of use or storage stability of an active ingredient.

For preparation of capsules 2 gms of sodium alginate was blended in 98 ml of water for 4 to 5 minutes to dissolve the chemical by thoroughly stirring with magnetic stirrer. Solution containing known number of nematodes was added in alginate

solution to get desired infective juvenile inoculum level. In a separate flask 1.47gm of calcium chloride dehydrate was mixed in 100ml of water. Then nematodes in alginate solution were loaded into a separating funnel and were dropped in calcium chloride solution so that calcium alginate capsules are formed with nematode infective juvenile inside (Plate 10). Allowed the capsules to remain in cold water for 20 to 30 minutes. Then the capsules were washed with distilled water to remove the excess calcium chloride.

### **3.3 EFFECT OF ENVIRONMENTAL FACTORS ON ENTOMO-PATHOGENIC NEMATODES**

Nematodes in natural habitat are buffered from environmental extremes such as temperature, moisture, relative humidity and light (Kaya, 1990). Nematode survival and pathogenicity was mainly depend on environmental factors.

#### **3.3.1 Effect of temperature on survival of entomopathogenic nematodes**

Soil temperature influences the performance of many important physiological processes in EPNs such as locomotion,

growth and reproduction (Prosser, 1973). It decides successful exploitation of nematodes as bio-control agents against soil dwelling insects (Bedding, 1982).

For studying the effect of temperature on survival of entomopathogenic nematodes, ten petridishes of 9 cm diameter were taken and sterilized in an autoclave at 120°C. In each petridish 1000 IJS of *S. carpocapsae* and *H. indica* were taken in 10 ml of water and kept in incubator by maintaining different temperatures i.e. 20, 25, 30 and 35°C. Observations on survival or mortality of the IJS were recorded at 24 hours interval. For this one ml of each replicate was taken after thorough shaking of nematode solution and then observed under microscope.

### ***3.3.1.1 Effect of temperature on pathogenicity of entomopathogenic nematodes***

For testing the influence of nematode pathogenicity in causing disease to *G. mellonella*, tissue-culture petriplates containing six wells were used. In to these wells final instar larvae of the test insect were released one for each well separately on to a filter paper spread inside the well (Plate 11). Then the larva inoculated

with nematode infective juveniles @ 60 IJS per larva in all the five wells, sixth well with larva and with out nematodes treated as check. All the petriplates were kept in incubator at different temperatures i.e. 20, 25, 30 and 35°C. Larval mortality was recorded over a period of 96 hrs with an interval of 24 hrs for both *S. carpocapsae* and *H. indica*.

### **3.3.2 Effect of relative humidity on survival of entomopathogenic nematodes**

For knowing the effect of relative humidity on survival of entomopathogenic nematodes, sterilized petridishes of 9 cm diameter were taken. In each petridish 10000 IJS of *S. carpocapsae* and *H. indica* were taken in 10 ml water and kept in relative humidity chamber at different humidity levels i.e. 50, 60, 70 and 80 per cent at room temperature (25±2°C). Observations on mortality of nematodes were recorded over a period of 24 hrs with an interval of 4 hours, for which 1 ml of each replicate was taken after thorough shaking of nematode solutions. Control was maintained out side the RH chamber with nematode solution.

### **3.3.2.1 Effect of relative humidity on pathogenicity of entomopathogenic nematodes**

For testing the influence of RH on nematode pathogenicity in causing disease to final instar larvae of *G. mellonella*, tissue culture petriplates containing six wells were used. Into these wells final instar larva of the test insect was released one for each well separately on to a filter paper spread inside the well. On the insect larva nematode IJS of *S. carpocapsae* and *H. indica* were released separately at the rate of 60 IJS per well. Sixth well with larva and without nematodes treated as check. All the petriplates were kept in relative humidity chamber at different RH levels of 50, 60, 70 and 80 per cent. Observations on larval mortality was recorded 48 hours after inoculation.

### **3.3.3 Effect of UV radiation on survival of entomopathogenic nematodes**

The ultraviolet (UV) portion of solar radiation was generally regarded as the most destructive abiotic factor affecting the persistence of entomopathogens (Ignoffo and Hostetter, 1977). Activity of the entomopathogenic nematodes also influenced by UV

radiation, affecting their movement, survival and further infection of insect pests. For knowing the effect of UV radiation an experiment was carried out under laboratory conditions by using *G. melloelle* larvae. For observing the influence of UV radiation on nematode survival, sterilized petridishes of 9 cm diameter were taken into which 10000 IJS of *S. carpocapsae* and *H. indica* into 10 ml solution were released and placed in a laminar air flow chamber under UV light (at average of 254 nm wave length). Petridishes with infective juveniles kept out side the chamber at room temperature maintained as check. Observations were recorded on nematode survival over a period of 120 minutes with an interval of 15 minutes for which 1 ml from each replicated nematode solution was taken after thorough shaking of nematode suspension. Data was recorded on live and dead nematodes for a period of two hours and experiment was replicated three times.

#### **3.3.3.1 Effect of UV radiation on pathogenicity of entomopathogenic nematodes**

For knowing the effect of UV infection process on *G. mellonella*, the UV exposed IJS of *S. carpocapsae* and *H. indica*

at the rate of 60 IJS per larva were inoculated for different time intervals. Then efficacy in causing disease to the test insect was observed. These petridishes with insect and IJS were incubated at room temperature. Data was recorded on number of dead or diseased larvae 48 hrs after inoculation.

#### **3.3.4 Effect of pH on survival of entomopathogenic nematodes**

Influence of pH on nematode survival was one of the most important constraints of environmental factors.

For knowing the effect of pH on survival of *S. carpocapsae* and *H. indica*, freshly harvested nematodes were used. For this solutions of different pH levels of 5, 6, 7, 8 and 9 were prepared by mixing different concentrations of H<sub>2</sub>SO<sub>4</sub> and NaOH. Test was carried out in 9 cm diameter petriplates where into each petriplate 10ml suspension containing 10000 IJS of nematodes were taken in each pH solution and kept at room temperature (25 ± 2°C). Observations were made on survival of IJS upto 20 days with an interval of five days for each observation.

#### **3.3.4.1 Effect of pH on pathogenicity of entomopathogenic nematodes**

For testing the influence of pH on nematode pathogenicity in causing disease to *G. mellonella*, glass petriplates were used. Each petriplate was filled with soil and solution of different pH levels i.e. 5, 6, 7, 8, 9 was poured. Then final instar larvae were released and inoculated with infective juveniles of two nematodes species i.e. *S. carpocapsae* and *H. indica* into separate petriplates. Larval mortality was recorded over a period of 96 hrs with an interval of 24 hrs. This experiment was replicated three times.

#### **3.3.5 Effect of soil moisture on survival of entomopathogenic nematodes**

To observe the effect of soil moisture on survival of the infective juveniles of the two nematode species i.e. *S. carpocapsae* and *H. indica* studies were conducted under laboratory conditions at room temperature. For this sandy loam soil was used and different moisture levels i.e. 5, 10, 15, 20 and 25 per cent were prepared by estimating through dry weight basis method by adding required quantity of water. Then the each soil sample of different moisture level was taken into petriplate and 1.5 ml of

nematode solution containing 1000 IJS were released. Per cent survival of the nematodes was recorded by sieving the soil sample with distilled water. Data was recorded over a period of 120 hrs with an interval of 24 hrs. This experiment was replicated 3 times for both *S. carpocapsae* and *H. indica*.

#### **3.3.5.1 Effect of soil moisture on pathogenicity of entomopathogenic nematodes**

For knowing the influence of soil moisture on nematode pathogenicity against *G. mellonella*, ten larvae were released into glass petriplates separately for each moisture level i.e. 5, 10, 15, 20 and 25 per cent and then inoculated with nematode infective juveniles @ 60 per larva. Larval mortality was recorded over a period of 96 hrs with an interval of 24 hrs. This experiment was replicated three times for both *S. carpocapsae* and *H. indica*.

#### **3.3.6 Effect of soil type on pathogenicity of entomopathogenic nematodes**

Soil was the most suitable target habitat for nematodes as it provides shelter and protection to them offering a safe environment from environmental extremes for establishment and

recycling. Studying of interaction between different soil types and nematode persistence under laboratory conditions provides basic information on optimal and threshold conditions for nematode persistence in soil.

To know the impact of soil type on nematode pathogenicity, experiment was conducted by using sand, sandy loam and black soils. Test details consisted of glass tubes filled with sterilized sand at three different levels i.e. 5, 10 and 15 cm in which a final instar larva of *G. mellonella* was placed at the bottom of each tube. Later to each tube 0.5 ml of nematode suspension containing 60 IJS were added on to the top of each column. Then the glass tubes were plugged with cotton and were kept upside down for observing infection. Mortality data was recorded on number of larvae showing symptoms at 48 hours after inoculation. The same procedure was followed in both sandy loam and black soils at the levels of 5, 10 and 15 cm. Experiment was done using both *S. carpocapsae* and *H. indica* at different depths of soils with the replication.

### **3.4 EVALUATION OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF IMPORTANT LEPIDOPTERAN PESTS**

Entomopathogenic nematodes have been reported as potential agents in reducing the insect pests especially the soil pests. However, larval populations of *Helicoverpa armigera*, *Spodoptera litura* and *Papilio demoleus*, move and pupate in the soil where they get chance of infection by entomopathogenic nematodes. For this purpose attempts were made to observe the infection and disease development on these pests by using entomopathogenic nematodes.

#### **Maintenance of insect cultures**

Different instar larvae of *H. armigera* were collected from different crops and were reared individually in separate plastic vials by providing sufficient food. After entering into pupation these were separated out and kept in plastic troughs for adult emergence. The emerged adults were released in a cage for egg laying. Red gram twigs with flowers were placed in glass cage for egg laying. Daily fresh twigs were kept and eggs were collected. The neonate larvae emerged were reared individually in separate vials. From this culture uniform aged larvae were used for experiment.

For maintaining the culture of *Spodoptera litura*, egg masses collected from groundnut fields were kept in plastic troughs. The neonate larvae emerged were reared on castor leaves by providing fresh feed daily.

For rearing *P. demoleus* larvae, different instar larvae collected from citrus orchard were reared by providing sufficient feed. After entering into pupation, these were separated out and kept in plastic troughs for adult emergence. Emerged adults were released in a cage for egg laying. Tender citrus twigs were placed in glass cage as egg laying sites. Daily, fresh twigs were kept and eggs were collected. The neonate larvae emerged were reared in separate troughs and used for experiment.

#### **3.4.1 Pathogenicity of *S. carpocapsae* / *H. indica* on**

##### ***Helicoverpa armigera***

To test the pathogenicity of *S. carpocapsae*, *H. indica* on *Helicoverpa armigera*, tissue culture petriplates containing six wells were used. Into these wells final instar larvae of the test

insect were released one for each well separately on to filter paper spread inside the well. Then the insect larva inoculated with nematode infective juveniles in different concentrations i.e. 20, 40, 60 and 80 IJS in 200 µl distilled water per well sixth well larvae with distilled water without nematodes treated as check. Observations on the mortality of larvae were recorded upto 96 hours with 24 hours interval. Experiment was replicated three times at each concentration at room temperature ( $25 \pm 2^{\circ}\text{C}$ ).

#### **3.4.2 Pathogenicity of *S. carpocapsae* / *H. indica* on *Spodoptera litura***

For testing the pathogenicity of *S. carpocapsae*, *H. indica* on *Spodoptera litura*, final instar larvae were released one for each well separately in tissue culture petriplates and IJS were released at different concentrations i.e. 20, 40, 60 and 80 IJS in 200µl suspension per each well. Sixth well with larvae without nematodes treated as check. Observation on the mortality of larvae were recorded by seeing the colour changes of larvae upto four days at 24 hours interval. Experiment was replicated three times.

### **3.4.3 Pathogenicity of *S. carpocapsae* / *H. indica* on *Papilio demoleus***

To test the pathogenicity of *S. carpocapsae* and *H. indica* on citrus butterfly *P. demoleus*, final instar larvae were released one for each well separately in tissue culture petriplates and IJS were released in different concentrations i.e. 20, 40, 60 and 80 IJS in 200µl suspension for each well. The larva released in this sixth well of the tray was treated as check i.e. without exposing to nematodes. Observations on the morality of larvae were recorded for four days at 24 hours interval, experiment was replicated three times for each concentration.

Median lethal dosages (LD<sub>50</sub>) were calculated for 24, 48, 72 and 96 hrs of exposure, on the basis of probit analysis.

$$LD_{50} = \frac{5 - \bar{Y}}{b}$$

$$\bar{Y} = \frac{\sum WY}{\sum W}$$

$$\bar{X} = \frac{\sum WX}{\sum W}$$

X = Log values of dosages

Y = Probits of mortality percentage

W = Weighing coefficient

Apart from LD<sub>50</sub> values, the median lethal time (LT<sub>50</sub>) for late instar larvae were calculated by converting time into log values and per cent kill in to probit kill at all inoculum levels *viz.*, 20, 40, 60 and 80 IJS / larva.

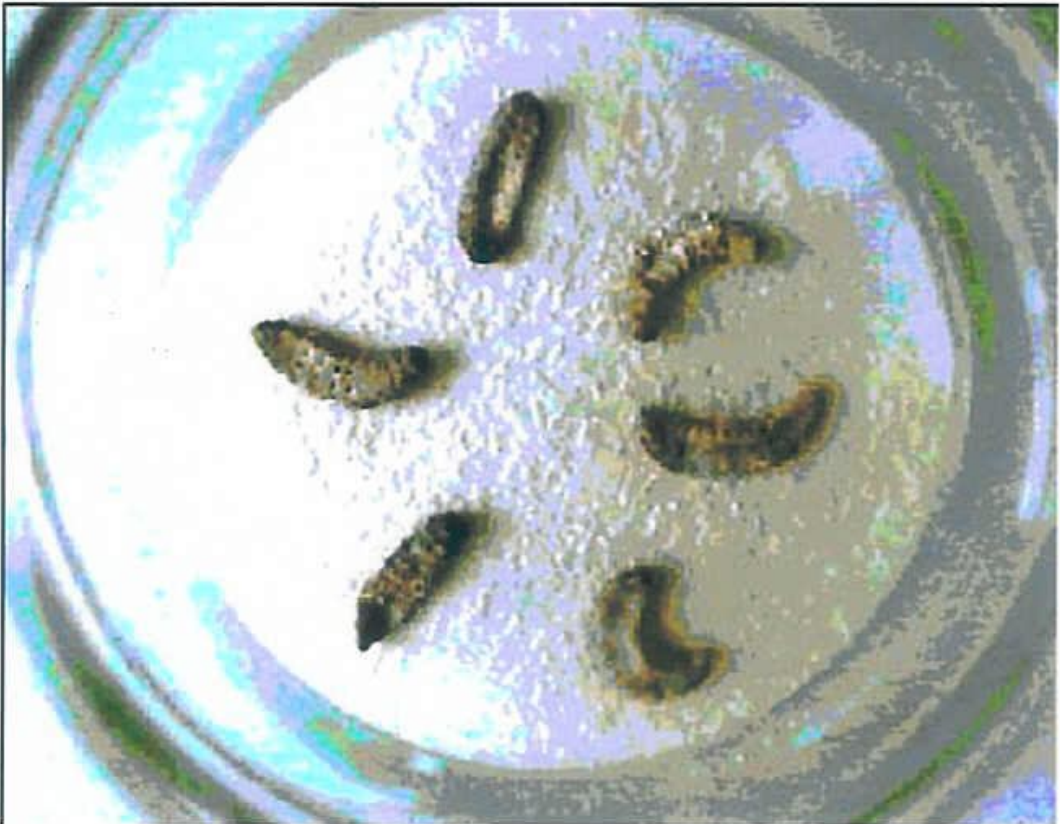
Statistical analysis of the data was carried out as per the procedures of Reghupathy and Dhamu (2001).

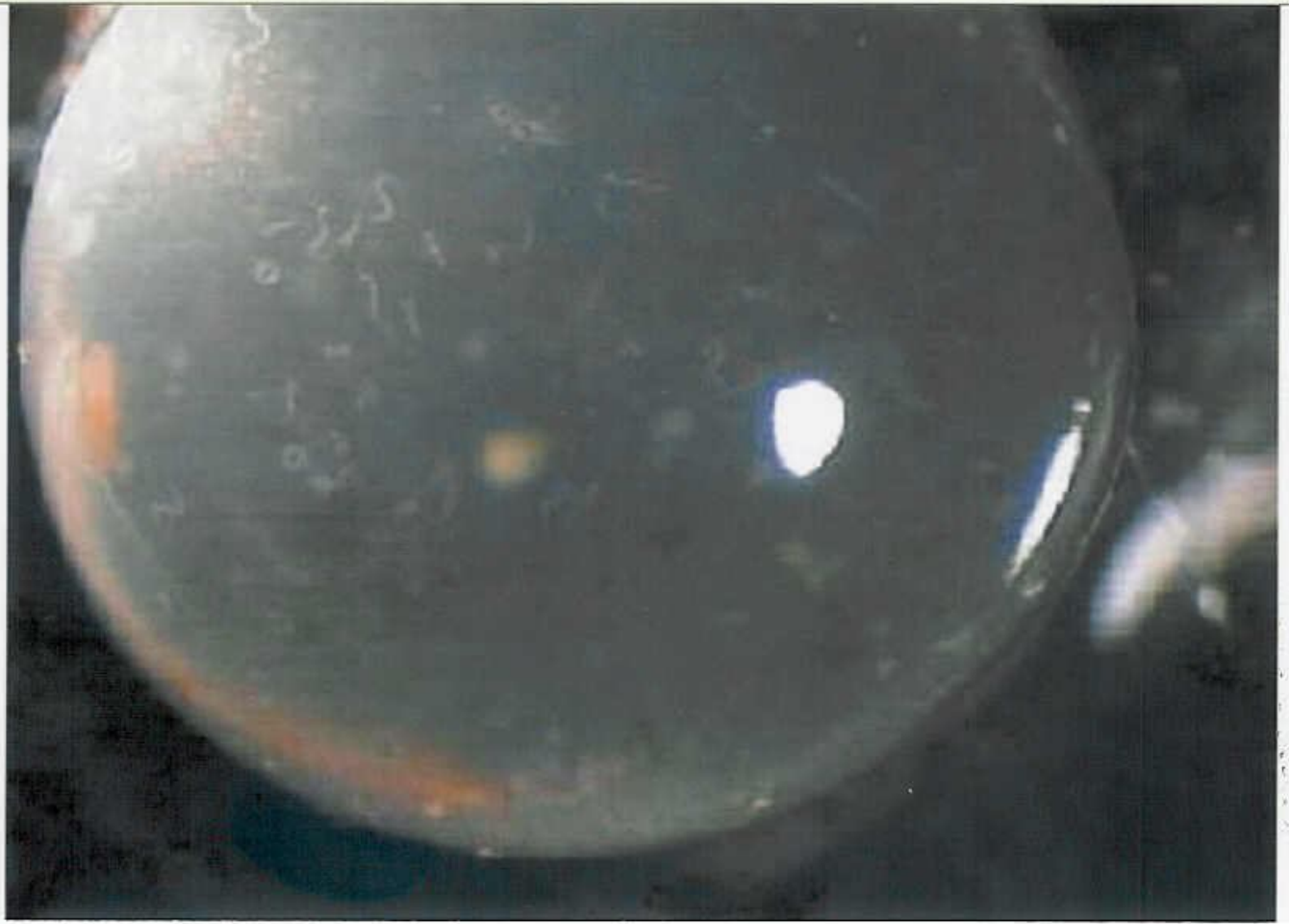












## **CHAPTER – IV**

### **RESULTS**

The results of the investigations carried out on survey for isolation of entomopathogenic nematodes, their mass multiplication, influence of environmental factors on survival and pathogenicity of entomopathogenic nematodes and evaluation of entomopathogenic nematodes (EPNs) for their efficacy against important lepidopteran pests, are presented in this chapter under different headings.

#### **4.1 SURVEY FOR ISOLATION OF ENTOMOPATHOGENIC NEMATODES**

Survey was carried out during January – June 2005 for isolation of entomopathogenic nematodes. A total of 135 samples were collected from different agricultural crops of different regions. Nematodes could not be recovered from any of the samples (Table 3).

## **4.2 IDENTIFICATION AND MASS MULTIPLICATION OF ENTOMOPATHOGENIC NEMATODES**

No nematodes were identified from the soil samples that have been collected in and around Tirupati region and Mahanandhi region.

### **4.2.1 Nematode culture**

Nematode cultures obtained from P.D.B.C were used by using *G. mellonella* larvae as the host insect for maintenance of nematode culture. These were further used for experimentation.

### **4.2.2 Maintenance of laboratory hosts**

#### **4.2.2.1 Maintenance of greater wax moth (*G. mellonella*)**

Culture of the greater wax moth larvae was maintained continuously by providing artificial diet and final instar larvae were used for mass multiplication.

#### **4.2.2.2 Maintenance of rice moth (*Corcyra cephalonica*)**

Culture was continuously maintained on broken sorghum grains fortified with groundnut seeds under laboratory conditions and final instar larvae were used for mass multiplication.

#### **4.2.2.3 Maintenance of silk worm culture (*Bombyx mori*)**

Silk worm culture was maintained continuously on mulberry leaves. Mass multiplication was carried out on fourth instar larvae.

#### **4.2.3 Mass multiplication of entomopathogenic nematodes on *Galleria mellonella* at different levels of infective juveniles**

Studies were carried out on multiplication of the two entomopathogenic nematodes *S. carpocapsae* and *H. indica* using *Galleria mellonella* as the host insect, by inoculating with 40, 60, 80 and 100 infective juveniles per larva. Results revealed that as the inoculum level increase, there was a gradual increased in progeny production of both *Steinernema carpocapsae* and *Heterorhabditis indica*. In the case of *S. carpocapsae*, a progeny population of (1.49 lakh) was obtained at the lowest inoculum level of 40 juveniles and 2.30 lakhs with 60 infective juveniles. But there was a decrease in the production of juveniles when the inoculum was increased to 80 and 100 infective juveniles per larva recording a progeny yield of 2.17 and 1.85 lakh juveniles of *S. carpocapsae* per larva. Hence, it was clear that a single larva of

*G. mellonella* could result in the production of a maximum number of 2.30 lakh juveniles when 60 infective juveniles were inoculated to single larva.

In the case of *H. indica*, there was an increase in the progeny production as the level of infective juveniles increased from 40 to 60 and 80, which resulted in a progeny production of 3.52, 4.10 and 4.34 lakh juveniles respectively. However, when 100 infective juveniles were inoculated, progeny production was reduced to 4.04 lakh juveniles per larva. Hence, it was clear from the present studies that a single *Galleria mellonella* larva could be infected only with 60 juveniles of *S. carpocapsae* and 80 juveniles of *H. indica* for mass multiplication. More than 60 infective juveniles of *S. carpocapsae* and 80 infective juveniles of *H. indica* when infected to the larva of *G. mellonella*, it could result in low progeny production (Table 4 and Fig.1).

#### **4.2.4 Yield of entomopathogenic nematodes from larvae of different sizes of greater wax moth, *Galleria mellonella***

Studies were carried out for finding the suitable size of the final instar larva of *Galleria mellonella* due to variation in their

body weight. Experiment conducted with larvae of different sizes i.e. small (body length of 10-12 mm and weight of 150 mg), medium (body length of 14-16mm and weight of 240 mg) and large (body length of 20 – 22 mm and weight of 350 mg), revealed that as the size of the larva was more, it could result in higher progeny production of both *S. carpocapsae* and *H. indica*. In the case of *S. carpocapsae*, there was only a difference of 6,920 juveniles in production between small and medium sized larvae were used as hosts and a difference of 21,060 and 14,140 juveniles could be recorded between small and large to medium and large sized larvae of *Galleria mellonella*. Hence, it can be concluded that only large sized larvae resulted in higher progeny of 1,02,400 juveniles per larvae and either small or medium sized larvae resulted in equivalent progeny yield.

In the case of *H. indica* there was a drastic difference in the production of juveniles when different sized larvae were used. The data revealed that small sized larva yielded about 1,06,000 juveniles, medium sized larva yielded about 1,48,400 juveniles and large sized larva yielded about 2,01,560 juveniles. It was clear from the results that a difference in juveniles number ranging

from 42,400 to 53,160 could be recorded by using different sized larvae of *G. mellonella* for multiplying *H. indica*. In general, as the weight of the larva was increasing, progeny that could be produced from the larva also increased which was more evident in the case of *H. indica* compared to *S. carpocapsae* (Table 5).

#### **4.2.5 Progeny production of EPN isolates from different insect hosts**

Experiment was conducted to find out alternate host insect for mass multiplication of *S. carpocapsae* and *H. indica* other than *G. mellonella* by using *Corcyra cephalonica* and *Bombyx mori*. Results presented in the Table 6 and Fig.2 revealed that *G. mellonella* yielded in about a total of 2.30 lakh progeny per larva followed by *C. cephalonica* about 1.83 lakh juveniles. Lowest of 1.13 lakh progeny was recorded in the case of *B. mori*. Similar pattern of progeny yield was recorded with respect to *H. indica* i.e. *G. mellonella* yielded a maximum progeny of 4.10 lakhs and *C. cephalonica* about 2.52 lakhs. The lowest population of 2.13 lakhs was recorded when *B. mori* was used for mass multiplication. It was clear from the present results that *G. mellonella* was the best host that could be used for mass

production of *S. carpocapsae* and *H. indica*. Either *C. cephalonica* or *B. mori* could be used for production of *H. indica* as both the larvae yielded approximately equal number of juveniles as compared to maximum production by *Galleria*.

#### **4.2.6 Harvesting of nematodes**

Observations made on behaviour of EPNs revealed that the two nematodes showed variation in behaviour. In the case of *S. carpocapsae*, the emerged infective juveniles were grouped at a single place, where as in case of *H. indica* the emerged juveniles dispersed in the entire petriplate individually.

#### **4.2.7 Preparation of entomopathogenic nematode juveniles into capsule formulation**

Alginated capsules of the two species *S. carpocapsae* and *H. indica* were prepared. These capsules measured about 4.23 mm in diameter with pale white colour. These capsules of *S. carpocapsae* were stored at 10-12°C and those of *H. indica* were stored at 5 – 10°C.

### **4.3 EFFECT OF ENVIRONMENTAL FACTORS ON ENTOMOPATHOGENIC NEMATODES**

Experiments were carried out under laboratory conditions by using the two genera of entomopathogenic nematodes i.e. *S. carpocapsae* and *H. indica* for observing the effect of environmental factors like temperature, relative humidity and U.V. radiation. Studies were also extended to observe the influence of pH, soil moisture and soil texture on pathogenicity and the results obtained are presented.

#### **4.3.1 Effect of temperature on survival of entomopathogenic nematodes**

Experiments conducted for observing the influence of different temperature levels i.e. 20, 25, 30 and 35°C on survival of the infective juveniles of the nematodes of the two species i.e. *S. carpocapsae* and *H. indica*, revealed that with increasing level of temperature there was gradual decrease in survival of the infective juveniles of *S. carpocapsae*. Data presented in the Table 7 and Fig.3 showed that, 25°C temperature was the optimum and effective temperature, showing maximum of 95.82 per cent survival of the juveniles. At 20°C, a mean of 84.57 per cent

survival could be observed. With increase in temperature to 30°C, there was only 77.06 per cent survival. At maximum temperature of 35°C, only a minimum of 37.14 per cent survival of the infective juveniles was recorded. Hence, a significant effect of temperature could be recorded on survival of the infective juveniles of *S. carpocapsae*.

Further, the data recorded on survival of the infective juveniles of *S. carpocapsae* at different time intervals, revealed that at 25°C, a maximum of 100 per cent survival of the infective juveniles was recorded upto 48 hrs followed by 96.49 per cent upto 74 hrs, 92.95 per cent after 96 hrs and 89.66 per cent after 120 hrs. When the temperature was raised to 30°C, 90.82 per cent juveniles could survive after 24 hrs and only 61.93 per cent after 120 hrs. Whereas at a maximum of 35°C, only 60.89 per cent survival after 24 hrs and minimum of 6.16 per cent after 120 hrs was recorded. When the temperature was decreased to 20°C, the percentage of survival of infective juveniles ranged from 94.73 to 73.79 per cent by 24 to 120 hrs.

In the case of *H. indica*, results presented in the Table-7 revealed that a temperature of 20°C was optimum, showing maximum of 91.66 per cent survival. Where as at 25°C, 88.89 per cent survival was recorded. When temperature was raised to 30°C, only 77.95 per cent survival could be resulted. A minimum of 40.30 per cent survival was recorded when temperature was raised to 35°C. Data on survival per cent of infective juveniles survived at different time intervals revealed that at 25°C, a maximum of 100 per cent survival of the infective juveniles was recorded after 24 hrs and 86.45 per cent after 120 hrs. When temperature was raised to 30°C, 91.09 per cent juveniles could survive after 24 hrs and only 64.13 per cent after 120 hrs. At a maximum of 35°C, it could result 63.47 per cent survival of juveniles after 24 hrs and a minimum of 10.95 per cent after 120 hrs. When the temperature was decreased to 20°C, it resulted in a maximum of 98.16 per cent survival of the juveniles after 24 hrs and 86.45 per cent after 120 hrs.

It was clear from the above results that 25°C temperature was the optimum temperature for *S. carpocapsae* and 20°C for *H. indica* at which infective juveniles should be very active and

showed high survival rate. 35°C was the most unfavourable temperature for both the nematode species at which activity and survival of infective juveniles was hampered.

#### **4.3.1.1 Effect of temperature on pathogenicity of entomopathogenic nematodes**

Results pertaining to the influence of different temperatures on the infectivity or pathogenicity against *Galleria mellonella* larvae with both *S. carpocapsae* and *H. indica* are presented in the Table 8 and Fig.8. Data revealed that temperature had a significant effect on infectivity of the test insect, inoculated with *S. carpocapsae* which has exhibited the disease symptoms. Out of four temperatures, 25°C was reported as the suitable temperature which resulted in 100 per cent mortality of the larvae showing complete discolouration of the larval body followed by its death. Similarly incubation of larvae inoculated with the juveniles of EPN maintained at 20°C also resulted in mortality of 97.18 per cent where, the effect is almost similar to that of 25°C. At 30°C, on an average 82.48 per cent mortality of the larvae was recorded. However, 35°C was proved to be the most unfavourable temperature for the infectivity by *S. carpocapsae* which resulted in about only 31.05 per cent infection.

Data pertaining to the influence of temperature on infection by *H. indica* to the larvae of *Galleria mellonella* revealed that both 20 and 25°C temperature could result in 99 to 100 per cent mortality of the test insect. Larvae inoculated with *H. indica* when incubated at temperatures of 20 and 25°C, exhibited the symptoms of red colouration of the body equally. This is followed by 83.14 per cent mortality observed at 30°C. However, 35°C was proved to be the most unfavourable temperature which resulted in only 30.03 per cent mortality.

It could be concluded from the above results that both *S. carpocapsae* and *H. indica* were able to infect the larvae efficiently at 25°C followed by 20°C. However, among the two nematodes, *H. indica* could result in a maximum of 99.11 per cent compared to 97.18 per cent by *S. carpocapsae*. A temperature of 35°C was found to affect infection process by EPNs and recorded as most unsuitable temperature (Fig.4).

Results presented in the Table 8 with respect to the time required for causing mortality of *G. mellonella* larva, revealed that 20 to 25°C temperatures were found to be favourable for causing maximum per cent disease within 24 hours. Whereas, when the larvae were maintained at 30°C and infected with *S. carpocapsae* it resulted in 87.20 per cent mortality. The highest temperature of 35°C resulted in a lowest rate of 48.30 mortality within 24 hours. After observing of the data on mortality of the infected larva over a period of 24, 48, 72 and 96 hrs times, it was clear that at 25°C, infectivity continued upto four days recording cent per cent mortality. At 35°C, very low infectivity resulting in low mortality of 31.05 per cent of the larva. However, 30°C temperature has recorded almost similar per cent mortality of the *G. mellonella* larvae even up to four days and 20°C could result in 93.1 to 98.13 per cent mortality due to infection by *S. carpocapsae*.

Similar observations were recorded with respect to infection by *H. indica* in causing mortality of *G. mellonella* larvae, the juveniles *H. indica* at 25°C were found to be active even upto 96

hours causing 100 per cent mortality. This is followed by 20°C which has showed similar pattern of causing 97.40 to 99.30 per cent mortality. At 30°C temperature, though initial mortality of 88.06 per cent was recorded immediately after 24 hours, gradually it was declined to 80.40 per cent by fourth day at a slow rate. 35°C is the most unfavourable temperature affecting the juveniles' infectivity resulting in only 40.9 per cent mortality after 24 hours period and further reduced to 16.9 per cent by 96 hours after inoculation with *H. indica*.

It was clear from the results (Fig.4) that at 25°C, juveniles were active for four days resulting in 100 per cent mortality of the test insect followed by 20°C which has shown almost similar effect. When the temperature was raised to 30°C, a slight decrease in the activity of the juveniles of the nematodes could be observed. The maximum temperature i.e. 35°C was said to be the most unfavourable temperature for the activity of both *S. carpocapsae* and *H. indica* affecting their infection process resulting in low mortality of less than 50.0 per cent of the *G. mellonella* larvae.

#### **4.3.2 Effect of relative humidity on survival / mortality of entomopathogenic nematodes**

Experiment conducted for observing the influence of different levels of humidity on survival of infective juveniles expressed in terms of per cent mortality. Results revealed that increased relative humidity improved the activity of juveniles of both *S. carpocapsae* and *H. indica*. The data presented in Table 9 and Fig.5 showed that maximum relative humidity of 80 per cent, on an average 79.72 per cent survival of the infective juveniles was recorded. 70 per cent relative humidity is required for survival of more than 50 per cent of the juveniles, whereas at 60 per cent relative humidity, only a mean of 22.48 per cent survival could be observed. At lowest relative humidity of 50 per cent only a minimum of 4.17 per cent survival of the juveniles was observed after 24 hrs. Hence, a significant effect was observed on survival / mortality of the infective juveniles of both *S. carpocapsae* due to variation in relative humidity.

Further, the data recorded on the time taken for causing mortality of the infective juveniles when maintained at different relative humidity levels revealed that occurrence of minimum relative humidity of 50 per cent, could result in 76.30 per cent in 4 hours 100 per cent mortality within 12 hrs. 60 per cent relative humidity could in 41.17 per cent mortality after 4 hrs followed by 68.20 per cent mortality after 8 hrs and ultimately 92.1 per cent and cent percent mortality by 12 and 16 hrs. Hence, it could be concluded from the above results that no nematode could able to survive after 12 hrs, when the relative humidity was about 50 to 60 per cent. When the relative humidity level was raised to 70 per cent, nearly 50 per cent of juveniles i.e. 48.30 per cent could survive even upto 24 hrs. After 4 hrs, it resulted in survival of 69.4 per cent, after 8 hrs. 67.3 per cent, after 12 hrs 64.30 per cent, after 16 hrs 61.4 per cent followed by 57.70 per cent after 20 hrs of exposure. Maximum relative humidity of 80 per cent resulted in more than 71.20 per cent survival of juveniles even after 24 hrs. In total, significant effect of relative humidity could be observed on survival of infective juveniles of *S. carpocapsae*.

Similar pattern of results were observed when the infective juveniles of *H. indica* were observed for their survival maintained at different levels of relative humidity i.e. 50, 60, 70 and 80 per cent. It was clear from the results that on an average lowest relative humidity level of 50 per cent resulted in only 7.0 per cent survival of juveniles after 24 hrs followed by 60 per cent showing 19.48 per cent ultimately resulting in only 93.0 and 80.52 per cent mortality of *H. indica*. Maximum relative humidity of 80 per cent resulted in survival of nearly 80 per cent juveniles (79.50%) with 20.50 per cent mortality of *H. indica* even after 24 hrs. 70 per cent relative humidity could resulted 63.65 per cent survival with 36.34 per cent mortality of the juveniles after 24 hrs with 63.65 per cent survival. However, maximum relative of 80 per cent was recorded as the most suitable for the resulting in maximum survival of 79.80 per cent and with a low mortality rate of 20.50 per cent of *H. indica* (Table 9).

#### **4.3.2.1 Effect of relative humidity on pathogenicity of entomopathogenic nematodes**

Results pertaining to the influence of relative humidity on two species of nematodes i.e. *S. carpocapsae* and *H. indica* in causing pathogenicity against *G. mellonella* larvae were presented in Table 10 and Fig.6. These revealed that with increasing level of relative humidity, there was significant increase in larval mortality inoculated with *S. carpocapsae*. A minimum of 50 per cent relative humidity resulted in less than 50 per cent (41.69%) larval mortality. At 60 per cent, relative humidity, 46.59 per cent larval mortality was recorded. When relative humidity was increased to 70 per cent, it could result in 65.02 per cent larval mortality. At a maximum relative humidity of 80 per cent, high larval mortality of 78.10 per cent was recorded.

Observations made on the time interval required for getting mortality to *Galleria* larva revealed that at maximum relative humidity of 80 per cent, there was highest larval mortality of 88.16 by 24 hrs and 66.43 per cent after 96 hrs. There was no much difference in causing mortality of the test insect when infected larvae was inoculated either at 50 of 60 per cent relative

levels, which resulted in almost equal per cent mortality ranging from 58.12 per cent to 61.96 per cent after 24 hrs. Incubation of *Galleria* larvae at 70 per cent, resulted in 73.15 per cent larval mortality after 24 hrs, 69.26 per cent after 48 hrs, 60.73 per cent after 72 hrs and 56.94 per cent after 96 hrs.

Results also revealed when the IJS of *H. indica* were inoculated to *G. mellonella* larva at about 80 per cent moisture level, on an average of 81.58 per cent mortality could be recorded. Whereas at 70 per cent relative humidity 69.79 per cent, 53.51 per cent mortality at 60 per cent relative humidity and less than 50.00 per cent (58.46%) larval mortality at 50 per cent relative humidity level was recorded. However, a maximum of 92.16 per cent larval mortality was recorded after 24 hrs of inoculation of *H. indica* at maximum relative humidity of 80 per cent. 70 per cent relative humidity has resulted in 80.73 per cent larval mortality after 24 hrs. Lowest relative humidity of 50 per cent has showed a mortality of 62.39 per cent after 24 hrs, 54.86 per cent after 48 hrs, 41.25 per cent after 72 hrs and 35.34 per cent after 96 hrs.

It was clear from the above results that 80 per cent relative humidity was very effective for both the nematode species in causing high larval mortality of the *Galleria* larva. As the humidity level decreased, the percentage mortality was also decreased.

#### **4.3.3 Effect of U.V Radiation on the survival /mortality of entomopathogenic nematodes.**

Observations made on the effect of U.V radiation on survival/mortality of EPNs presented in the Table 11 and Fig.7 revealed that more than 80.0 per cent of juveniles could survive only up to 15 minutes of U.V light exposure (254 nm). And if the juveniles were exposed to U.V radiation for a long period of 120 minutes, it could result in complete mortality of the juveniles. In general, it was observed that when the juveniles of either *S. carpocapsae* or *H. indica* were exposed to U.V. radiation for a longer period, it could result in complete death of the juveniles affecting their activity. However, when juveniles were exposed for 30 minutes to U.V light, only about 69.90 per cent of *S. carpocapsae* and 58.83 per cent of *H. indica* juveniles have survived. Exposure for more than 45 minutes to U.V. radiation could result in survival of less than 50 per cent of juveniles of both

nematodes. After 60 and 75 minutes of exposure, it resulted in still more cent per cent mortality of both *S. carpocapsae* and *H. indica* was recorded 120 minutes of exposure to U.V. light. Finally, it could be concluded from the results that among the two nematodes, *H. indica* was found to be more sensitive to U.V radiation than *S. carpocapsae* this could result in 100.0 per cent mortality after 90 minutes of U.V light exposure in *H. indica*, which occurred after 120 minutes of U.V light exposure in the case of *S. carpocapsae*. Ultimately, it was clear that *S. carpocapsae* could comparatively resist U.V radiation for longer period than *H. indica* (Table 11).

#### **4.3.3.1 Effect of U.V radiation on pathogenicity of entomopathogenic nematodes.**

Experimental results pertaining to the effect of U.V radiation on pathogenicity of *G. mellonella* larva by using *S. carpocapsae* and *H. indica* presented in Table 12 and Fig.8 revealed that, among the two groups of nematodes, *S. carpocapsae* was more virulent in causing mortality of the test insect when it was infected juveniles that were exposed to U.V radiation even for 75 minutes compared to *H. indica* where the juveniles of *H. indica* when exposed to U.V radiation for more than one hour (60 minutes)

could not able to infect the *Galleria* larva. Exposure for a minimum period of 15 minutes to U.V light, juveniles of both *S. carpocapsae* and *H. indica* were able to cause 100 per cent infection in the test insect. If the juveniles were exposed to U.V. radiation for 30 minutes, both the nematode species could result in about 93.40 to 80.30 per cent mortality followed by 80.21 and 48.42 per cent after 45 minutes and 46.45 and 26.63 per cent after 60 minutes of exposure in the case of *S. carpocapsae* and *H. indica* respectively. However, *H. indica* could not infect the *Galleria* larva when the juveniles were exposed to U.V radiation for more than 60 minutes but *S. carpocapsae* could result in about 20.78 per cent mortality at the same condition. It was concluded from the above results that there was significant effect of time interval of U.V radiation on disease causing ability of the juveniles of both *S. carpocapsae* and *H. indica*. However, among the two species *S. carpocapsae* could tolerate U.V. radiation for longer than *H. indica*.

#### **4.4.4 Effect of pH on mortality/survival of entomopathogenic nematodes**

Results pertaining to the effect of pH on survival of the two nematode species i.e. *S. carpocapsae* and *H. indica* were presented

in the Table 13 and Fig.9 there was a gradual increase in percentage of survival of the infective juveniles of *S. carpocapsae* with increasing pH i.e. from 5 to 7. As the pH was increased, there was a decrease in survival of the juveniles. However the effects of both pH levels of 6 and 7 were on par with each other. At a pH of 7, maximum of 48.55 per cent survival of the infective juveniles was recorded and at 6 there was slight decrease in the survival of the infective juveniles recorded as 41.93 per cent, followed by 33.93 per cent at pH 5. With increasing pH from 7 to 9 there was a significant decrease in survival of infective juveniles which was recorded 24.60 per cent at a pH 8 and 16.80 per cent at 9 pH (Table 13).

Data regarding the percentage survival of the infective juveniles of different days revealed that a pH of 7 resulted in maximum of 70.60 per cent survival of the infective juveniles i.e. 70.60 upto 5 days followed by 51.30 per cent upto 10 days, 40.90 per cent upto 15 days and 31.40 per cent upto 20 days. pH of 6 was resulted in a decrease of survival percentage 60.7 to 10.30 per cent by twenty days. Where as at pH 5 maximum of 52.40 per cent survival was recorded after 5 days and 17.40 per cent after 20

days. Whereas with increasing pH levels i.e. at 8 there was 39.9 per cent survival of the juveniles after 5 days and 10.5 per cent after 20 days. At maximum level of pH 9 only 28.60 per cent survival was observed after 5 days and a minimum of 5.70 per cent survival of infective juveniles was recorded after 20 days.

Similar pattern of results were observed with *H. indica* expressed in terms of survival percentage at different pH levels i.e. 5, 6, 7, 8 and 9. It was clear that pH level of 7 has resulted in a maximum of 66.40 per cent survival after 5 days and 55.12 per cent survival of the juveniles was recorded after twenty days, followed by 68.60 per cent at pH 6 after 5 days and 47.42 per cent at lowest pH of 5 there was 61.60 per cent survival of the juveniles after 5 days and 18.57 per cent after 20 days. When the pH was raised to 8 there was a gradual decrease in percentage survival of the juveniles, which was ranging from 40.7 per cent to 15.70 per cent by 5 to 20 days. survival percentage was further decreased to 34.03 after 5 days and 9.9 per cent after 20 days was observed when pH was further raised to 9.

From the above results, it was clear that a pH level of 7 was the most suitable level for maximum survival of the two nematode species i.e. *S. carpocapsae* and *H. indica* to have at least more than 50.0 per cent survival of the infective juveniles.

#### **4.3.4.1 Effect of pH on pathogenicity of entomopathogenic nematodes**

When *G. mellonella* larva in soil medium was infected with *S. carpocapsae* IJS maintained in water at different pH levels. Results presented in the Table 14 and Fig.10 revealed that at neutral pH of 7.0, there could be an average mortality of 78.60 per cent of the test insect. When the pH was reduced to 6.0, it resulted in about 58.64 per cent and further at a pH of 5.0, there was about 49.44 per cent mortality, when pH was increased to 8.0, there was a drastic reduction in per cent mortality which was recorded as 52.83 per cent followed by 40.27 at maximum pH level of 9. Observations made on the time required for causing mortality of *Galleria* larva revealed that, under neutral pH condition of the soil, there was maximum 96.43 per cent mortality of the test insect after 24 hrs. There was no much difference in causing mortality of the test insect when the soil medium was watered with

water having pH levels of 6.0 and 8.0 which resulted in almost equal per cent mortality ranging from 75.3 per cent to 78.12 percent. Similar effect was recorded when the two pH levels i.e. 5.0 and 9.0 showing 60.32 to 65.37 per cent mortality could be recorded after 24 hours. With reference to influence of *H. indica* on mortality of *G. mellonella* larva when the soil medium was maintained at different pH levels, the result revealed that *H. indica* showed same influence on the test insect as *S. carpocapsae*. At neutral pH of 7.0, on an average a maximum of 82.06 per cent mortality was recorded. Where as at a pH of 6.0, 65.04 per cent and at 5.0, 55.84 per cent mortality was recorded. When the pH was increased from 7.0 to 8.0, there was a drastic decrease in infection of the larvae i.e. 57.17 percent, and further reduced to 48.34 per cent when the pH was increased to 9.0.

A maximum of 98.19 per cent mortality was recorded after 24 hrs of inoculation of *H. indica* at neutral pH i.e. 7.0. However, a slight decrease of pH to 6.0 or increase to 8.0 resulted in the mortality of test insect to range of 82.13 and 88.43 per cent. At lowest pH of 5.0 there was 56.37 per cent and at highest pH of 9, 51.37 per cent mortality of larva was recorded.

It was made clear from the above results that nematode activity and its effect in causing the mortality of the test insect remained unaltered with the neutral pH of 7.0. A slight deviation in pH level from 7.0 to 5.0 or from 7.0 to 9.0 could result in causing more than 50.0 per cent mortality of *G. mellonella* larvae by both *S. carpocapsae* and *H. indica*.

#### **4.3.5 Effect of soil moisture on survival of entomopathogenic nematodes.**

Results pertaining to the effect of soil moisture of different levels i.e. 5, 10, 15, 20 and 25 per cent on survival of the two nematodes species i.e. *S. carpocapsae* and *H. indica* are presented in Table 15 and Fig.11 revealed that there was a gradual increase in survival percentage of the infective juveniles upto the field capacity (15%), and there was a decrease in survival of the juveniles with increasing soil moisture from 15 to 20 per cent. Data showed that a minimum of 5 per cent soil moisture was required for survival of more than 50 per cent of the juveniles, at 20 per cent moisture level, about 45.25 per cent survival of the juveniles was reported. At maximum soil moisture of 25 per cent,

on an average 39.58 per cent survival of the juveniles was recorded. Maximum survival percentage of 78.62 per cent was observed at moisture level of 15 per cent followed by 63.99 per cent at 10 per cent.

Data recorded on per cent survival of injective juveniles of *S. carpocapsae* with regard to time interval revealed that at favourable soil moisture level of 15 per cent, maximum survival of 96.45 per cent after 24 hrs, 84.13 per cent after 48 hrs, 78.09 per cent after 72 hrs, 70.13 per cent after 96 hrs and 64.37 per cent after 120 hrs was observed. The lowest moisture level of 5 per cent resulted in survival of 72.13 per cent after 24 hrs, 63.47 per cent after 48 hrs, 53.49 per cent after 72 hrs, 44.31 per cent after 96 hrs and 52.17 per cent after 120 hrs. However, maximum soil moisture of 25 per cent also resulted in only 56.19 per cent after 24 hrs, 46.39 per cent after 48 hrs, 40.49 per cent after 72 hrs, 30.27 per cent after 96 hrs and 24.59 per cent after 120 hrs.

It could be concluded that 15 per cent moisture level resulted in a survival of more than 50.0 per cent of juveniles of *S. carpocapsae* even after 120 hrs or five days.

Similar pattern of results were observed when the infective juveniles of *H. indica* were observed for their survival at different levels of soil moisture i.e. 5, 10, 15, 20 and 25 per cent. It was clear from the results that at lowest soil moisture level of 5 per cent, survival of the infective juveniles was decreased from 76.17 to 38.18 per cent by 24 hrs to 120 hrs followed by 10 per cent moisture showing 80.23 per cent survival after 24 hrs and a minimum survival of 53.42 per cent after 120 hrs. at optimum level i.e. at 15 per cent soil moisture, there was high percentage survival of the juveniles recorded as 97.13 per cent after 24 hrs, 88.12 per cent after 48 hrs, 79.43 per cent after 72 hrs, 70.32 per cent after 96 hrs and 66.47 per cent after 120 hrs. However, when the soil moisture was increased to 20 per cent, there was significant decrease in survival percentage of IJS i.e. 72.17 per cent after 24 hrs and 34.13 per cent after 120 hrs. Maximum soil moisture level i.e. 25 per cent has recorded about 58.13 per cent survival of IJS after 24 hrs and 31.45 per cent after 120 hrs.

#### **4.3.5.1 Effect of Soil moisture on pathogenicity of entomopathogenic nematodes**

Results obtained pertaining to the effect of soil moisture on nematode pathogenicity against *G. mellonella* infected with EPNs presented in the Table 16 and Fig.12 revealed that at lowest of 5 per cent moisture level, less than 50 per cent (37.66 per cent) mortality of the larvae was observed. when the moisture level was increased to 10 percent, mortality was significantly increased to 52.16 per cent and at 15 per cent moisture level, a maximum of 75.97 per cent mortality was recorded. At about 20 per cent moisture level, there was a significant reduction in per cent mortality of *Galleria* which was recorded as 46.92 per cent followed by 35.88 per cent at 25 per cent moisture level.

Observations made on the time interval required for causing mortality of *Galleria* larva, revealed that at 15 per cent moisture level a maximum larval mortality of 86.97 per cent after 24 hrs period, followed by 80.13 per cent after 48 hrs, 72.47 per cent after 72 hrs, and 64.32 per cent after 96 hrs. At 10 per cent moisture level larval mortality was recorded as 65.73 per cent after 24 hrs and 39.13 per cent after 96 hrs. At 20 per cent moisture

level, percentage larval mortality was decreased from 60.13 per cent to 32.96 per cent within 96 hrs. There was no much difference in causing mortality of the test insect at harvest of 5 per cent and maximum of 25 per cent moisture levels which resulted in almost equal per cent mortality ranging from 50.16 per cent to 53.43 per cent after 24 hrs and 20.76 per cent to 23.92 per cent after 96 hrs.

With reference to influence of *H. indica* on mortality of *G. mellonella* larva at different moisture levels, results revealed that, at 15 per cent moisture level, on an average a maximum of 79.99 per cent mortality was recorded. Incubation of larvae at 5 per cent soil moisture level resulted in about 45.24 per cent and at 10 per cent, 57.59 per cent mortality was recorded. When the moisture level was increased to 20 per cent there was a decrease in mortality of the larva i.e. 56.78 per cent and further reduced to 43.78 per cent when the moisture was increased to 25 per cent.

A maximum of 91.13 per cent mortality was recorded after 24 hrs of inoculation of *H. indica* at 15 per cent soil moisture level and 70.34 per cent after 96 hrs. There was no significant

difference in per cent larval mortality at 5 and 25 per cent moisture levels ranging from 56.13 per cent to 60.69 per cent after 24 hrs and 30.64 to 30.37 after 96 hrs. At 10 per cent moisture level, there was 69.13 per cent mortality after 24 hrs followed by 61.76 per cent after 48 hrs 56.34 per cent after 72 hrs 43.13 per cent after 96 hrs was observed. At 20 per cent moisture level, maximum larval mortality of 64.96 per cent and minimum of 39.74 per cent after 96 hrs was observed.

It was clear from the above results that nematode activity and its effect in causing the mortality of the test insect was high at 15 per cent moisture level and was low at 25 per cent moisture levels of soil.

#### **4.3.6 Effect of soil type on pathogenicity of entomopathogenic nematodes**

Results pertaining to the experiment conducted on effect of soil type i.e. sandy, sandy loam and black soils on the pathogenicity of *Galleria* larva inoculated with *S. carpocapsae* and *H. indica* are presented in Table 17 and Fig.13. The data revealed that after 48 hrs of inoculation of the juveniles of *S. carpocapsae*

in black soil, there was maximum mortality of 72.60 per cent of test insect followed by sandy loam with 67.33 per cent and 58.2 per cent by sandy soil and sandy with only 58.2 per cent mortality. Similar trend was observed with respect to *H. indica* which has resulted in a maximum of 77.28 per cent mortality in black soil followed by 67.86 in sandy loam. Lowest of 64.00 per cent mortality of the host insect was recorded in sandy soil. It could be made clear from the above results that among the three types of soils, black soil has favoured the nematode activity followed by pathogenicity of *Galleria* irrespective of the nematode species. Data recorded on the influence of depth of the nematode inoculation to the test insect, it was clear from the table that as the depth of soil increased, it resulted in less mortality of the test insect, in both the nematodes. When the juveniles were released on to the top 5 cm soil, *S. carpocapsae* caused about 77.33 per cent and *H. indica* about 90.7 per cent mortality of the insect larvae. Where as when the juveniles were released at 10 cm depth, *S. carpocapsae* could infect and cause mortality of *Galleria* only up to an extent of 68.6 where as *H. indica* up to 80.83 per cent. However lowest mortality of 37.66 per cent with *H. indicia* and 55.70 per cent with *S. carpocapsae* could be recorded. When the

juveniles were released in 15cm depth of soil. It was clear from the results that among the two species, *H. indica* has more host searching activity compared to *S. carpocapsae*.

#### **4.4 EVALUATION OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF IMPORTANT LEPIDOPTERAN PESTS**

To test the pathogenicity of two entomopathogenic nematodes, *S. carpocapsae* and *H. indica* against lepidopteran pests i.e. *Helicoverpa armigera*, *Spodoptera litura* and *Papilio demoleus* experiments were carried out under laboratory conditions and results obtained were documented in terms of the percentage mortality of the larvae of the test insect.

##### **4.4.1 Pathogenicity of *S. carpocapsae* and *H. indica* on *H. armigera***

It was observed that *S. carpocapsae* infected larvae of *H. armigera* turned to light brown colour (Plate 12) and *H. indica* infected larvae turned to dark brown colour (Plate 13).

Results indicated that as the level of infective juveniles increased, there was significant increase in mortality of *H. armigera*. However, nearly 50 per cent mortality of the larvae was observed at an inoculum levels of 60 infective juveniles per larva resulting in 48.20 per cent immediately after 24 hrs. Whereas lowest level of 20 juveniles resulted in 13.20 per cent and even highest inoculum of 80 infective juveniles also resulted in only 54.30 per cent mortality of *H. armigera* larva (Table 18).

It was also clear from the results that low level of infective juveniles i.e. 20 juveniles per larva could not result in more than 29.40 per cent mortality of *H. armigera* even after four days i.e. 96 hrs. Similarly when 40 infective juveniles were inoculated, it resulted in 54.60 per cent and 60 IJS resulted in a maximum of 66.90 per cent by 96 hrs after inoculation. It could be concluded that *S. carpocapsae* when inoculated at a moderate level of 60 IJS per larva could cause on an average more than 50.0 per cent mortality. However, highest inoculum level of 80 IJS could cause an average mortality of 66.65 per cent of *H. armigera*.

Results presented in Table 18a indicated that mortality of *H. armigera* was directly related to the dosage of infective juveniles. To cause 50.0 per cent mortality of the host insect within 24 hrs, it required about 69.31 IJS. Similarly a dosage of 58.20 was necessary to cause mortality of the larvae within 48 hrs, followed by 46.99 and 35.24 IJS to result in 72 and 96 hrs respectively..

With regard to the median lethal time required for 50 per cent mortality of late instar of *H. armigera*, results were presented in Table 18b. As the level of infective juveniles increased, there was a significant decrease in time required for causing 50 per cent mortality of the test insect. The lowest level of 20 IJS required about 154.10 hrs or 6 days for causing death of 50 per cent larvae and the maximum of 80 IJS required only 23.55 or approximately one day for the same. Similarly 60 IJS took 33.34 hrs i.e. less than one and half day for causing mortality of 50 per cent larvae. Whereas 40 IJS comparatively took long time i.e. 91.41 hrs (less than four days) for causing 50 per cent mortality. It was clear from the results that a lethal dose of 58.20 IJS and a lethal time of 33.34 hrs was required for causing 50 per cent mortality of the test insect larvae.

Results presented in the Table-19 revealed that insect mortality was directly correlated to the dosage of infective juveniles of *H. indica*. At the lowest dosage of 20 IJS, there was a mean death of 22.53 per cent and at 40 IJS, 40.65 per cent mortality of *H. armigera* could be recorded. However, more than 50 per cent (LD<sub>50</sub>) i.e. 57.43 per cent mortality of *H. armigera* was recorded only at an inoculum level of 60 IJS per larva. At about maximum dosage of 80 IJS per larvae there was only an average 67.95 per cent mortality of the insect was observed.

Observations on the time required for the death of *H. armigera* at different dosages of *H. indica* (Table-19 ) revealed that 20 IJS could result in a maximum of 30.20 per cent mortality even after 96 hrs. Where as 40 IJS resulted in 54.80 per cent mortality of *H. armigera* after 4 days. However, with a minimum dosage of 60 IJS per larva resulted in 50.30 per cent mortality immediately after 24 hrs. Similar result of 56.20 per cent mortality of *H. armigera* larva was recorded when 80 IJS of *H. indica* were used.

The results on median lethal dose and lethal time required for 50 per cent kill of *H. armigera* revealed that the LD<sub>50</sub> of *H. indica* was 58.60 IJS per insect recorded after 24 hrs. If it has to occur within 2 days a single larva required about 51.40 IJS as LD<sub>50</sub>. For killing the larva of *H. armigera* in 72 hrs, the LD<sub>50</sub> was 44.77 IJS and it was 37.30 IJS per insect if the mortality take 96 hrs. Similarly the LT<sub>50</sub> was recorded as only 21.58 hrs causing 50.0 per cent mortality of *H. armigera* larva, when maximum number of 80 IJS were inoculated. When a dosage of 60 IJS was used, it took about 33.50 hrs. The lowest dosage of 20 and 40 IJS recorded the LT<sub>50</sub> as in a range of 92.40 to 140.30 hrs i.e. nearly 4 to 6 days for causing 50.0 per cent mortality of *H. armigera* (Table 19a & 19b) .

#### **4.4.2 Pathogenicity of *S. carpocapsae* and *H. indica* on *Spodoptera litura***

Results pertaining to the influence of *S. carpocapsae* on fifth instar larva of *S. litura* are presented in the Table-20, revealed that as the number of infective juveniles increased, there was a significant increase in mortality of the larva. At minimum dosage of 20 IJS per insect, resulted in an average mortality of 23.95 per

cent was recorded followed by 40.65 per cent at 40 IJS and 46.75 per cent mortality when 60 IJS of *S. carpocapsae* were inoculated per larva. Maximum dosage of 80 IJS per larva has resulted in more than 50.0 per cent mortality of (54.93%) of *S. litura*. Similarly at the lowest dosage of 20 IJS, maximum of 38.90 per cent mortality could be recorded after 96 hrs. Whereas maximum of 80 IJS resulted in about 26.70 per cent mortality of *S. litura* within 24 hrs and 54.60 per cent within 48 hrs. Similar pattern of causing mortality of *S. litura* was observed, when 60 IJS were inoculated to the larva of *S. litura*, by the end of 96 hrs after inculcation. Sixty and 80 IJS could result in causing mortality upto an extent of 64.80 to 73.20 per cent of 96 hrs.

The LD<sub>50</sub> value for 50.0 per cent mortality of *S. litura* with *S. carpocapsae* was recorded as a maximum of 140.3 IJS, if the death of insect has to result in 24 hrs and it was 59.02 IJS, for mortality to occur within 48 hrs. An average of 43.15 IJS were required to cause 50 per cent mortality of *S. litura* within 72 hrs. Similarly at a dosage of 28.84 IJS per larvae, it took about 96 hrs

for the same (Table 20a). The  $LT_{50}$  value was recorded as 42.95 hrs when maximum of 80 IJS of *S. carpocapsae* were inoculated per larva. Similarly 54.08 hrs when 60 IJS and 71.61 hrs when 40 IJS were used for causing 50 per cent death of the larvae of *S. litura*. When inoculum level of 60 IJS of *S. carpocapsae* were used against *S. litura* it took about 54.08 hrs and a maximum level of 80 IJS took only 42.95 hrs for resulting in 50.0 per cent mortality of *S. litura* (Table 20b).

Results pertaining to the pathogenicity of *H. indcia* against *S. litura* presented in the Table-21 revealed that insect mortality was directly related to the dosage of infective juveniles. At the lowest dosage of 20 IJS, there was an average mortality of 26.65 per cent and 41.95 per cent mortality at a dosage of 40 IJS. Approximately 50 per cent (49.93%) mortality of *S. litura* was recorded at an inoculum level of 60 IJS per larva. However, more than 50 per cent i.e. 56.95 per cent mortality of *S. litura* was recorded only at high inoculum level of 80 IJS per larva.

Observations on the time required for the death of *S. litura* at different inoculum levels of *H. indica* revealed that 20 IJS could result in a maximum mortality of 40.30 per cent after 96 hrs. Whereas 40 IJS resulted in 57.20 per cent mortality of *S. litura* after 4 days. However, with a dosage of 60 IJS per larva, there was 50.30 per cent mortality after 48 hrs. Similar result of 56.70 per cent mortality of *S. litura* larva was recorded when 80 IJS of *H. indica* were used. Significant influence of inoculum level of the nematode could be seen in causing disease in *S. litura* larvae.

The results on median lethal dose and lethal time required for 50 per cent kill of *S. litura* revealed that the LD<sub>50</sub> of *H. indica* for *S. litura* was 140.3 IJS per insect recorded after 24 hrs. If the mortality has to occur within 48 hrs, a single larva required about 59.02 IJS. For causing 50.0 per cent mortality of *S. litura* larva in 72 hrs, the LD<sub>50</sub> was only 42.95 IJS. When a dosage of 60 IJS was used, it took about 54.08 hrs. The lowest dosage of 20 and 40 IJS recorded the LT<sub>50</sub> as in a range of 71.61 to 15.18 hrs i.e. nearly 3 to 6 days for causing 56.0 per cent mortality of *S. litura* (Table-21a & 21b).

#### **4.4.3 Pathogenicity of *S. carpocapsae* and *H. indica* on *Papilio demoleus***

It was observed that *S. carpocapsae* infected larvae of *P. demoleus* turned to light brown colour (Plate 14) and *H. indica* infected larvae turned to dark brown colour (Plate 15).

Results pertaining to the influence of *S. carpocapsae* on fifth instar larvae of *P. demolous* are presented in the Table 22. As when the number of infective juveniles was increased, there was a significant increase in mortality of the larva. At minimum dosage of 20 IJS per insect it resulted in an average mortality of 27.65 per cent, followed by 41.95 per cent at 40 IJS. However more than 50 per cent i.e. 75.73 per cent mortality at 60 IJS level and 74.83 at 80 IJS level was recorded. At the lowest dosage of 20 IJS, a maximum of 41.20 per cent mortality could be recorded even after 96 hrs. At maximum inoculum level of 80 IJS, about 54.30 per cent mortality of *P. demoleus* could be recorded even with in 24 hrs and 75.27 per cent mortality after 48 hrs. When 60 IJS were inoculated, 48.20 per cent mortality was recorded after 24 hrs and

73.10 per cent after 48 hrs, by the end of fourth day after inoculation. However, 60 and 80 IJS took about 96 hrs for causing mortality of *P. demoleus* to an extent of 96.00 to 96.40 per cent.

The LD<sub>50</sub> value for causing 50.0 per cent mortality of *P. demoleus* with *S. carpocapsae* was recorded as maximum of 92.11 IJS, if the death of insect has to result within 24 hrs. It was recorded as 41.69 IJS for causing mortality within 48 hrs. An average of 33.19 IJS were required to cause 50 per cent mortality of *P. demoleus* within 72 hrs. Similarly if IJS were inoculated @ 26.55 per larva, it took about 96 hrs (Table 22a). The LT<sub>50</sub> value was recorded as 21.63 hrs when maximum of 80 IJS of *S. carpocapsae* were inoculated. Similarly 44.46 hrs when 60 IJS and 73.45 hrs when 40 IJS were used for causing 50 per cent mortality of *P. demoleus*. It took maximum of 138.8 hrs at lowest dosage of 20 IJS to kill 50 per cent of the *P. demoleus* larvae (Table 22b).

Results presented in the Table 23 revealed that insect mortality was directly correlated to the dosage of infective juveniles of *H. indica*. At the lowest dosage of 20 IJS, there was a mean death of 28.55 per cent and at 40 IJS dosage, 43.30 per cent

mortality of *P. demoleus* could be recorded 76.45 per cent mortality at 60 IJS per larva. A maximum 80.63 per cent mortality of *P. demoleus* was recorded at inoculum level of 80 IJS per larva.

Observations on the time required for the causing 50 per cent mortality of *P. demoleus* at different dosages of *H. indica* (Table - 23) revealed that 20 IJS could result in a maximum mortality of 42.70 per cent only after 96 hrs. Whereas 40 IJS resulted in 57.50 per cent mortality after 96 hrs. However, a dosage of 60 IJS per larva resulted in 50.20 per cent mortality within 24 hrs. similar results of 58.70 per cent mortality of *P. demoleus* was recorded when 80 IJS of *H. indica* were used.

When IJS of *H. indica* were inoculated @ 20 and 40 IJS, it resulted in an average larval mortality of *P. demoleus* extending to a range of 28.55 and 43.30 per cent. However, when 60 IJS were inoculated, it resulted in more than 50.00 per cent mortality even within 24 hrs and also increased to 58.70 per cent when 80 IJS were inoculated. However, on an average, significant effect of time interval was observed irrespective of the nematode inoculum level.

The results (Table 23a) on median lethal dose of *H. indica* required for 50 per cent mortality of *P. demoleus* revealed that the LD<sub>50</sub> value was 59.31 IJS per larva recorded after 24 hrs. If the mortality has to occur within 48 hrs, a single larva required about 41.69 IJS. For killing the larva of *P. demoleus* in 72 hrs the LD<sub>50</sub> was 33.88 IJS and 26.12 per insect, if the mortality has to take 96 hrs. Similarly LT<sub>50</sub> value was recorded as 11.43 hrs for *P. demoleus* larva when a maximum number of 80 IJS were inoculated. At a dosage of 60 IJS, LT<sub>50</sub> was recorded as 20.30 hrs. The lowest dosage of 20 and 40 IJS recorded the LT<sub>50</sub> in a range of 76.49 to 129.5 hrs i.e. nearly 3 to 5 days for causing 50.0 per cent mortality of *P. demoleus* (Table 23b).

When comparison was made for the susceptibility of the three lepidopterous pests i.e. *H. armigera*, *S. litura* and *P. demoleus* to both *S. carpocapsae* and *H. indica*, it was clear that, among the three insect pests, *P. demoleus* was more susceptible recording a maximum mortality of 75.73 per cent by *S. carpocapsae* and 70.45 per cent by *H. indica*. This is followed by *H. armigera* showing 66.65 per cent by *S. carpocapsae* and 67.95

per cent by *H. indica*. However, among the two entomopathogenic nematodes, *H. indica* was said to be more virulent than *S. carpocapsae* in causing comparatively high mortality of all the three pests irrespective of the level of inoculum (Table 24 and Fig.14).

Observations made on the calculation of median lethal dose  $LD_{50}$  of infective juveniles of both *S. carpocapsae* and *H. indica* revealed that the  $LD_{50}$  varied in relation to insect pests. Data recorded on  $LD_{50}$  values at which 50 per cent mortality of test insect occurred after 24 hrs, revealed that in case of *H. armigera*, it was high i.e. 69.31 IJS for *S. carpocapsae* and 58.60 IJS for *H. indica*. Similar trend was observed against *P. demoleus* with 72.11 IJS as  $LD_{50}$  for *S. carpocapsae* and 59.31 IJS for *H. indica*. But *S. litura* showed different reaction in its susceptibility to the entomopathogenic nematodes i.e. the  $LD_{50}$  for *S. carpocapsae* was 95.5 IJS and for *H. indica*, it was 140.3 IJS per larva. In general similar trend of variation with  $LD_{50}$  was recorded irrespective of the time interval for the nematodes (Table 25 and Fig.15).

Data presented in the Table 25 and Fig.16 with reference to the lethal time  $LT_{50}$  required for causing 50 per cent mortality of the three lepidopteran insects i.e. *H. armigera*, *S. litura* and *P. demoleus* revealed that *P. demoleus* required very less time compared *H. armigera* and *S. litura*. As the inoculum level of infective juveniles increased, the  $LT_{50}$  value was decreased.

When maximum number of infective juveniles i.e. 80 per larvae were inoculated the  $LT_{50}$  was recorded as 21.63 hrs for *S. carpocapsae* and 11.43 hrs for *H. indica* for *P. demoleus* followed by 23.55 hrs *S. carpocapsae* and 21.58 hrs for *H. indica* for *H. armigera*. However, compared to these two insects, *S. litura* needed  $LT_{50}$  value of 42.95 hrs of *S. carpocapsae* and 31.05 hrs of *H. indica* for resulting in 50.0 per cent mortality of the test insect. At lowest inoculum of 20 IJS per larva, *P. demoleus* required  $LT_{50}$  value as 138.8 hrs for *S. carpocapsae* and 129.5 hrs for *H. indica*, followed by 154.1 hrs for *S. carpocapsae* and 140.3 hrs for *H. indica*, 151.8 hrs for *S. carpocapsae* and 170.2 hrs for *H. indica* for *S. litura* needed to cause 50 per cent mortality of the test

insect. At 40 infective juveniles level required 73.45 hrs for *S. carpocapsae* and 76.59 hrs for *H. indica* for *P. demoleus* followed by 91.41 hrs for *S. carpocapsae* and 92.40 hrs for *H. indica* for *H. armigera* and 71.61 hrs for *S. carpocapsae* and 94.15 hrs for *H. indica* for *S. litura* needed for causing 50 per cent mortality. From all the levels 60 infective juveniles were optimum level which took 44.46 hrs for *S. carpocapsae* and 20.30 hrs for *H. indica* for *P. demoleus*, 33.34 hrs for *S. carpocapsae*, 33.50 hrs for *H. indica* for *H. armigera* and 54.08 hrs for *S. carpocapsae* 55.21 hrs for *H. indica* for *S. litura* was recorded.

**Table 3 : Survey for isolation of entomopathogenic nematodes**

<b>Region</b>	<b>Number of soil samples</b>	<b>Type of soil</b>	<b>Nematode isolated</b>
Pacchikapalam	35	Black soils	--
Chandragiri	20	Red & Black soils	--
Tirumala Hills	20	Black soils	--
Karakambody	10	Red soils	--
RARS., Tirupati	20	Sandy loam soils	--
Mahanandi region	30	Black soils	--

**Table 4 : Mass multiplication of entomopathogenic nematodes on *G.mellonella*.**

Inoculum level IJs/Larva	Progeny yield infective juveniles in lakhs/larva	
	<i>S.carpocapsae</i>	<i>H.indica</i>
40	1.49	3.52
60	2.30	4.10
80	2.17	4.34
100	1.85	4.04
Mean	1.84	4.00

**Average of 10 larvae**

**Table - 5 : Yield of entomopathogenic nematodes from different sized larvae of *G.mellonella***

Size of the larvae (mm)	Mean weight of the larvae (mg)	Progeny yield IJs/lrvae
<i>S.carpocapsae</i>		
Small (10–12)	150	81,340
Medium (14–16)	240	88,260
Large (20–22)	350	1,02,400
<i>H.indica</i>		
Small (12–14)	140	1,06,000
Medium (14–16)	220	1,48,400
Large (20–22)	340	2,01,560

**Mean of 10 larvae**

**Table - 6 : Progeny production of EPN isolates from different insect hosts.**

Host insect	Progeny yield IJs/larva in lakhs	
	<i>S.carpocapsae</i>	<i>H.indica</i>
<i>Galleria mellonella</i>	2.30	4.10
<i>Corcyra cephalonice</i>	1.83	2.52
<i>Bombyx mori</i>	1.13	2.13
Mean	1.75	2.92

**Average of 10 larvae**

**Table - 22 : Pathogenicity of *S.carpocapsae* on *Papilio demoleus***

Nematode inoculum IJs / larva	% mortality of larvae after hours				Mean
	24	48	72	96	
20	18.40 (25.40)	21.30 (27.49)	29.70 (33.02)	41.20 (39.93)	27.65 (31.76)
40	20.30 (26.78)	42.10 (40.45)	49.00 (44.43)	56.40 (48.68)	41.95 (40.40)
60	48.20 (43.97)	73.10 (58.76)	85.20 (67.37)	96.40 (79.06)	75.73 (60.47)
80	54.30 (47.47)	75.27 (60.20)	73.77 (59.21)	96.00 (78.46)	74.83 (59.87)
Control	0.0	0.0	0.0	0.0	
<b>Mean</b>	28.24 (32.08)	42.35 (40.63)	47.53 (43.57)	58.00 (49.60)	

Values in parenthesis are angular transformed values.

	F	SEM	CD
Dosages	**	4.9307	6.4347
Time	**	4.2701	5.7458
Dosages X time	**	9.8614	13.2693

**Table – 22a : Susceptibility of *P.demoleus* larvae to *S.carpocapsae* at different time intervals**

Time interval (Hrs.)	LD <sub>50</sub> (IJs / larvae)	Regression equation	Heterogeneity
24	72.11	$1.389 + 1.94 x$	0.06
48	41.69	$0.631 + 2.696 x$	0.044
72	33.19	$0.392 + 3.03 x$	0.047
96	26.55	$0.328 + 3.28 x$	0.193

**Table – 22b : Time mortality response of *P.demoleus* larvae to different dosages of *S.carpocapsae***

IJs	LT <sub>50</sub> (Hrs.)	Regression equation	Heterogeneity
20	138.8	$2.753 + 0.953 x$	- 0.022
40	73.45	$2.07 + 1.571 x$	0.021
60	44.46	$1.4 + 2.552 x$	0.051
80	21.63	$1.997 + 2.25 x$	0.008

**Table - 23 : Pathogenicity of *H.indica* on *Papilio demoleus***

Nematode inoculum IJs / larva	% mortality of larvae after hours				Mean
	24	48	72	96	
20	19.30 (26.06)	22.10 (28.04)	30.10 (33.27)	42.70 (40.80)	28.55 (32.33)
40	22.30 (28.18)	44.50 (41.84)	48.90 (44.37)	57.50 (49.31)	43.30 (41.15)
60	50.20 (45.11)	74.30 (59.54)	86.30 (68.28)	95.00 (77.08)	76.45 (61.00)
80	58.70 (50.01)	77.50 (61.68)	89.10 (70.72)	97.20 (80.37)	80.63 (63.87)
Control	0.0	0.0	0.0	0.0	
Mean	30.1 (35.27)	43.68 (41.38)	50.88 (45.52)	58.48 (49.89)	

Values in parenthesis are angular transformed values.

	F	SEM	CD
Dosages	**	0.4963	1.4319
Time	**	0.4298	1.2401
Dosages X time	**	0.9926	2.8638

**Table – 23a : Susceptibility of *P.demoleus* to *H.indica* at different time intervals**

<b>Time interval (Hrs.)</b>	<b>LD<sub>50</sub> (IJs / larvae)</b>	<b>Regression equation</b>	<b>Heterogeneity</b>
24	59.31	1.45 + 1.95 x	0.066
48	41.69	1.08 + 2.42 x	0.151
72	33.88	0.64 + 2.85 x	0.21
96	26.12	1.12 + 2.74 x	0.355

**Table – 23b : Time mortality response of *P.demoleus* to different dosages of *H.indica***

<b>IJs</b>	<b>LT<sub>50</sub> (Hrs.)</b>	<b>Regression equation</b>	<b>Heterogeneity</b>
20	129.5	2.65 + 1.02 x	0.03
40	76.59	2.18 + 1.53 x	0.05
60	20.30	2.83 + 1.43 x	0.15
80	11.43	3.61 + 1.31 x	0.32

**Table - 21 : Pathogenicity of *H.indica* on *Spodoptera litura***

Nematode inoculum IJs / larva	% mortality of larvae after hours				Mean
	24	48	72	96	
20	16.20 (23.73)	20.50 (26.92)	29.60 (32.96)	40.30 (39.41)	26.65 (31.05)
40	17.90 (25.03)	43.10 (41.03)	49.60 (44.77)	57.20 (49.14)	41.95 (40.40)
60	25.40 (30.26)	50.30 (45.17)	57.60 (49.37)	66.40 (54.57)	49.93 (44.94)
80	28.90 (32.52)	56.70 (48.85)	66.40 (54.57)	75.80 (60.53)	56.95 (49.02)
Control	0.0	0.0	0.0	0.0	
Mean	17.68 (24.88)	34.12 (35.73)	40.64 (39.58)	47.94 (43.80)	

Values in parenthesis are angular transformed values.

	F	SEM	CD
Dosages	**	0.3983	1.1377
Time	**	0.3562	1.0176
Dosages X time	**	0.7965	2.2755

**Table - 21a : Susceptibility of *Spodoptera litura* to *H.indica* at different time intervals**

Time interval (Hrs.)	LD <sub>50</sub> (IJs / larvae)	Regression equation	Heterogeneity
24	140.3	$2.85 + 0.83 x$	-0.02
48	59.02	$2.15 + 1.61 x$	-0.07
72	43.15	$2.79 + 1.35 x$	0.08
96	28.84	$3.02 + 1.35 x$	0.15

**Table - 21b : Time mortality response of *Spodoptera litura* to *H.indica* at different dosages**

IJs	LT <sub>50</sub> (Hrs.)	Regression equation	Heterogeneity
20	151.8	$0.95 + 2.72 x$	0.086
40	71.61	$1.83 + 1.71 x$	0.010
60	54.08	$2.33 + 1.54 x$	0.118
80	42.95	$1.70 + 2.02 x$	0.012

**Table - 20 : Pathogenicity of *S.carpocapsae* on *Spodoptera litura***

Nematode inoculum IJs / larva	% mortality of larvae after hours				Mean
	24	48	72	96	
20	12.40 (20.62)	16.70 (24.12)	27.80 (31.82)	38.90 (38.59)	23.95 (29.33)
40	16.80 (24.20)	42.10 (40.45)	47.30 (43.45)	56.40 (48.68)	40.65 (39.64)
60	23.30 (28.86)	44.60 (41.90)	54.30 (47.47)	64.80 (53.61)	46.75 (43.17)
80	26.70 (31.11)	54.60 (47.64)	65.20 (53.85)	73.20 (58.82)	54.93 (47.81)
Control	0.0	0.0	0.0	0.0	
Mean	15.84 (23.42)	31.6 (34.20)	38.92 (38.59)	46.66 (43.11)	

**Values in parenthesis are angular transformed values.**

	<b>F</b>	<b>SEM</b>	<b>CD</b>
Dosages	**	0.4293	1.2264
Time	**	0.3840	1.0969
Dosages X time	**	0.8586	2.4528

**Table – 20a: Susceptibility of *Spodoptera litura* larvae to *S.carpocapsae* at different time intervals**

Time interval (Hrs.)	LD <sub>50</sub> (IJs / larvae)	Regression equation	Heterogeneity
24	95.50	$1.61 + 1.71 x$	- 0.009
48	65.16	$1.88 + 1.72 x$	0.003
72	45.50	$-7.59 + 7.60 x$	- 1.072
96	28.67	$3.32 + 1.56 x$	0.102

**Table - 20b : Time mortality response of *Spodoptera litura* to different dosages of *S.carpocapsae***

IJs	LT <sub>50</sub> (Hrs.)	Regression equation	Heterogeneity
20	170.2	$2.59 + 1.47 x$	0.011
40	94.15	$2.72 + 1.21 x$	0.008
60	55.21	$2.76 + 1.29 x$	0.005
80	31.05	$3.33 + 1.12 x$	- 0.07

**Table - 19 : Pathogenicity of *H.indica* on *Helicoverpa armigera***

Nematode inoculum IJs / larva	% mortality of larvae after hours				Mean
	24	48	72	96	
20	15.60 (23.26)	18.20 (25.25)	26.10 (30.72)	30.20 (33.34)	22.53 (28.32)
40	30.20 (33.34)	34.20 (35.79)	43.40 (41.27)	54.80 (47.75)	40.65 (39.64)
60	50.30 (45.17)	52.43 (46.38)	59.70 (50.59)	67.30 (55.12)	57.43 (49.26)
80	56.20 (48.56)	60.80 (51.24)	70.40 (57.04)	84.40 (66.74)	67.95 (55.55)
Control	0.0	0.0	0.0	0.0	
Mean	30.46 (33.52)	33.13 (35.12)	39.92 (39.17)	47.34 (43.45)	

**Values in parenthesis are angular transformed values.**

	<b>F</b>	<b>SEM</b>	<b>CD</b>
Dosages	**	0.4580	1.3083
Time	**	0.4096	1.1702
Dosages X time	**	0.9159	2.6166

**Table - 19a : Susceptibility of *Helicoverpa armigera* to *H.indica* at different time intervals**

Time interval (Hrs.)	LD <sub>50</sub> (IJs / larvae)	Regression equation	Heterogeneity
24	58.60	1.29 + 2.05 x	- 0.003
48	51.40	2.83 + 2.08 x	0.035
72	44.77	2.27 + 1.65 x	0.091
96	37.30	2.24 + 1.57 x	0.150

**Table - 19b : Time mortality response of *Helicoverpa armigera* to different dosages of *H.indica***

IJs	LT <sub>50</sub> (Hrs.)	Regression equation	Heterogeneity
20	140.30	2.71 + 0.88 x	0.005
40	92.40	3.04 + 1.00 x	- 0.028
60	33.50	3.90 + 0.71 x	0.007
80	21.58	3.36 + 1.23 x	0.403

**Table 11 : Effect of U.V.Radiation on the survival of entomopathogenic nematodes**

Time (Min)	<i>S.carpocapsae</i>		<i>H.indica</i>	
	Survival %	Mortality %	Survival %	Mortality %
15	80.62 (63.87)	19.38 (26.13)	81.80 (64.75)	18.20 (25.25)
30	69.90 (56.37)	30.10 (33.27)	58.53 (49.89)	41.47 (40.11)
45	40.07 (39.29)	59.93 (50.71)	39.90 (39.17)	60.10 (50.83)
60	30.83 (33.71)	69.17 (56.29)	25.27 (30.20)	74.73 (59.80)
75	19.47 (26.21)	80.53 (63.79)	16.30 (23.81)	83.70 (66.19)
90	14.73 (22.54)	85.27 (67.46)	8.37 (16.85)	91.63 (73.15)
105	10.87 (19.28)	89.13 (70.72)	0.00	100.00 (88.19)
120	0.00	100.00 (88.19)	0.00	100.00 (88.19)
Mean	33.31 (35.24)	66.69 (54.76)	28.77 (32.46)	71.23 (57.34)

**Values in parenthesis are angular transformed values.**

**Table 12 : Effect of U.V.Radiation on pathogenicity of entomopathogenic nematodes**

Time (Min)	<i>S.carpocapsae</i>	<i>H.indica</i>
	% mortality of <i>G.mellonella</i>	% mortality of <i>G.mellonella</i>
15	100.00 (88.19)	100.00 (88.19)
30	93.40 (75.11)	80.30 (63.65)
45	80.21 (63.58)	48.42 (44.03)
60	46.45 (42.99)	26.63 (31.05)
75	20.78 (27.13)	0.00
90	0.00	0.00
105	0.00	0.00
120	0.00	0.00
Mean	42.61 (40.74)	31.92 (34.39)

**Values in parenthesis are angular transformed values.**

SEM	1.31	1.01
SED	1.85	1.43
CD	3.92	3.04
CV	4.47	4.07



**Table 13 : Effect of p<sup>H</sup> on mortality / survival of entomopathogenic nematodes**

p <sup>H</sup>	% mortality of <i>S.carpocapsae</i> IJs after days					% mortality of <i>H.indica</i> IJs after days				
	5	10	15	20	Mean	5	10	15	20	Mean
5	37.60 *(52.40) #(37.82)	62.30 (37.70) (52.12)	71.80 (28.20) (57.92)	82.60 (17.40) (65.35)	63.58 (36.42) (52.89)	38.40 (61.60) (38.29)	64.60 (35.40) (53.43)	74.03 (25.97) (59.34)	81.43 (18.57) (64.45)	66.12 (33.88) (54.39)
6	30.30 (60.70) (33.40)	41.90 (58.10) (40.34)	61.20 (38.60) (51.47)	89.70 (10.30) (71.28)	55.78 (44.22) (48.33)	31.40 (68.60) (34.08)	40.60 (59.40) (39.58)	59.70 (40.30) (50.59)	78.61 (21.39) (62.44)	52.58 (47.42) (46.49)
7	29.40 (70.60) (32.83)	48.70 (51.30) (44.26)	59.10 (40.90) (50.24)	68.60 (31.40) (55.92)	51.45 (48.55) (47.29)	33.60 (76.40) (35.43)	38.90 (61.10) (38.59)	47.30 (52.70) (43.45)	59.70 (40.30) (50.59)	44.88 (55.12) (42.07)
8	60.10 (39.90) (50.83)	69.30 (30.70) (56.35)	82.70 (17.30) (65.42)	89.50 (10.50) (71.09)	75.40 (24.60) (60.20)	59.30 (40.70) (50.36)	68.60 (31.40) (55.92)	79.60 (20.40) (63.15)	84.30 (15.70) (66.66)	72.95 (27.05) (58.69)
9	71.40 (28.60) (57.67)	78.50 (21.50) (62.38)	88.60 (11.40) (70.27)	94.30 (5.70) (76.19)	83.20 (16.80) (65.8)	65.97 (34.03) (54.33)	74.60 (25.40) (59.74)	85.20 (14.80) (67.37)	90.10 (9.90) (71.66)	78.97 (21.03) (62.73)
Mean	47.76 (52.24) (43.74)	60.14 (39.86) (50.83)	72.68 (27.32) (58.50)	84.94 (15.06) (67.13)		44.53 (55.47) (41.84)	57.46 (42.54) (49.31)	69.17 (30.83) (56.29)	78.83 (21.17) (62.58)	

	F	SEM	CD	F	SEM	CD
p <sup>H</sup>	* *	0.4564	1.3039	* *	0.5862	1.6746
Time after days	* *	0.4082	1.1662	* *	0.5243	1.4978
p <sup>H</sup> x Time	* *	0.9129	2.6078	* *	1.1724	3.3491

\* Values in parenthesis are % survival of EPNs.

# Values in parenthesis are angular transformed values.

**Table - 17 : Effect of soil type on pathogenicity of entomopathogenic nematodes.**

<i>S.carpocapsae</i>					<i>H.indica</i>			
Type of the soil	% mortality of <i>G.mellonella</i> larvae after 48 hours			Mean	% mortality of <i>G.mellonella</i> larvae after 48 hours			Mean
	5 (cm)	10 (cm)	15 (cm)		5 (cm)	10 (cm)	15 (cm)	
Sandy	67.20 (55.06)	64.20 (53.25)	43.20 (41.09)	58.20 (49.72)	83.40 (65.96)	78.40 (62.31)	30.40 (33.46)	64.06 (53.19)
Sandy loam	73.30 (58.89)	68.40 (55.80)	60.30 (50.94)	67.33 (55.12)	90.40 (71.95)	80.97 (64.01)	32.20 (34.57)	67.86 (55.49)
Black	82.50 (65.27)	73.20 (58.82)	62.10 (52.0)	72.60 (58.44)	98.30 (82.51)	83.13 (65.73)	50.40 (45.23)	77.28 (61.55)
Mean	77.33 (61.55)	68.60 (55.92)	55.20 (47.98)		90.70 (72.24)	80.83 (64.01)	37.66 (37.88)	

**Values in parenthesis are angular transformed values.**

**Table 9 : Effect of Relative Humidity on mortality / survival of entomopathogenic nematodes.**

R.H level %	<i>S.carpocapsae</i>							<i>H.indica</i>						
	% mortality of IJs after hours						Mean	% mortality of IJs after hours						Mean
	4	8	12	16	20	24		4	8	12	16	20	24	
50	76.30 *(23.70) #(60.87)	99.70 (0.3) (86.86)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	95.83 (4.17) (78.17)	68.30 (31.70) (55.73)	89.70 (11.30) (71.28)	100.0 (0.0) (88.19)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	93.00 (7.0) (74.66)
60	41.17 (58.83) (39.93)	68.20 (31.80) (55.67)	92.10 (7.90) (73.68)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	83.58 (16.42) (66.11)	36.40 (63.60) (37.11)	64.30 (36.70) (53.31)	88.70 (11.30) (70.36)	93.70 (6.30) (75.46)	100.00 (0.0) (88.19)	100.00 (0.0) (88.19)	80.52 (19.48) (63.79)
70	30.60 (69.40) (33.58)	32.70 (67.30) (34.88)	35.20 (64.30) (36.39)	38.60 (61.40) (38.41)	42.30 (57.70) (40.57)	51.70 (48.30) (45.97)	38.43 (61.57) (38.29)	27.30 (72.70) (31.50)	30.10 (69.90) (33.27)	32.30 (67.70) (34.63)	38.40 (61.60) (38.29)	41.70 (59.30) (40.22)	48.30 (51.70) (44.03)	36.35 (63.65) (37.11)
80	14.13 (95.87) (22.06)	15.23 (84.77) (22.95)	21.10 (78.90) (27.35)	23.50 (76.50) (29.00)	24.90 (75.10) (29.93)	28.80 (71.20) (32.46)	21.28 (79.72) (27.49)	14.30 (85.70) (22.22)	15.70 (84.30) (23.34)	19.60 (80.40) (26.28)	22.70 (77.30) (28.45)	23.60 (76.40) (29.06)	27.27 (72.73) (31.50)	20.50 (79.50) (26.92)
Mean	40.43 (59.57) (39.47)	53.96 (46.04) (47.29)	62.10 (37.90) (52.00)	65.53 (34.47) (54.03)	66.80 (33.20) (54.82)	70.13 (29.87) (56.85)		36.58 (63.42) (37.23)	49.95 (50.05) (45.00)	60.15 (39.85) (50.83)	63.70 (36.30) (52.95)	66.30 (33.70) (54.51)	68.90 (31.10) (56.10)	

	F	SEM	CD	F	SEM	CD
RH	* *	0.2974	0.8410	* *	0.2744	0.7763
Mortality	* *	0.3642	1.0301	* *	0.3361	0.9507
RH x mortality	* *	0.7284	2.0601	* *	0.6723	1.9014

\* Values in parenthesis are % survival of EPNs.

# Values in parenthesis are angular transformed values.



**Table - 18 : Pathogenicity of *S.carpocapsae* on *Helicoverpa armigera*.**

Level of nematode inoculum IJs/ larva	Mortality of <i>H.armigera</i> (hours after infection)				Mean
	24	48	72	96	
20	13.20 (21.30)	16.40 (23.89)	24.30 (29.53)	29.40 (32.83)	20.83 (27.13)
40	28.40 (32.20)	34.30 (35.85)	42.30 (40.57)	54.60 (47.64)	39.90 (39.17)
60	48.20 (43.97)	50.30 (45.17)	55.60 (48.22)	66.90 (54.88)	55.25 (48.04)
80	54.30 (47.47)	59.70 (50.59)	69.20 (56.29)	83.40 (65.96)	66.65 (54.76)
Control	0.0	0.0	0.0	0.0	
Mean	28.82 (32.46)	32.14 (34.51)	38.28 (38.23)	46.86 (43.22)	

**Values in parenthesis are angular transformed values.**

	F	SEM ±	CD at 1%
Dosages	**	0.5502	1.5718
Time after hours	**	0.4921	1.4059
Dosages X Time	**	1.1004	3.1436

**Table -18a : Susceptibility of *H.armigera* to *S.carpocapsae* at different time intervals**

Time interval	LD <sub>50</sub> (IJs/Larva)	Regression equation	Heterogeneity
24	69.31	1.208 + 2.06 x	0.51
48	58.20	2.016 + 1.67 x	0.26
72	46.99	1.612 + 2.03 x	0.53
96	35.24	1.357 + 2.36 x	0.36

**Table -18b : Time mortality response of *H.armigera* to different dosage of *S.carpocapsae***

Level of inoculum (IJs/larva)	LT <sub>50</sub> (hours)	Regression equation	Heterogeneity
20	154.10	2.48 + 0.98 x	0.05
40	91.41	2.81 + 1.12 x	0.06
60	33.34	3.89 + 0.13 x	0.13
80	23.55	3.27 + 1.26 x	0.21

**Table 8 : Effect of temperature on pathogenicity of entomopathogenic nematodes**

Temperature (°C)	<i>S.carpocapsae</i>					<i>H.indica</i>				
	Mortality percentage after hours				Mean	Mortality percentage after hours				Mean
	24	48	72	96		24	48	72	96	
35	48.30 (44.03)	36.33 (37.05)	23.10 (28.73)	16.26 (23.81)	31.05 (33.90)	40.90 (39.76)	34.30 (35.85)	28.03 (31.95)	16.90 (24.27)	30.03 (33.21)
30	87.20 (69.04)	84.63 (66.89)	79.50 (63.08)	78.60 (62.44)	82.48 (65.27)	88.06 (69.82)	83.20 (65.80)	80.90 (64.09)	80.40 (63.72)	83.14 (65.73)
25	100.00 (89.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)
20	98.13 (82.08)	99.30 (85.2)	98.20 (82.29)	93.10 (74.77)	97.18 (80.37)	99.30 (85.20)	100.00 (88.19)	99.72 (86.86)	97.40 (80.72)	99.11 (84.56)
Mean	83.40 (65.96)	80.11 (63.51)	75.20 (60.13)	71.99 (58.05)		82.06 (64.97)	79.37 (63.01)	77.16 (61.48)	73.68 (59.15)	

Values in parenthesis are angular transformed values.

	F	SEM <sub>±</sub>	CD	F	SEM <sub>±</sub>	CD
Temperature	* *	0.4472	1.7362	* *	0.6824	1.9687
Time after hours	* *	0.3873	1.5036	* *	0.5910	1.7049
Temperature X Time	* *	0.8945	3.4725	* *	1.3648	3.9374

**Table 7 : Effect of temperature on the survival of entomopathogenic nematodes.**

Temperature (°C)	<i>S.carpocapsae</i>						<i>H.indica</i>					
	Survival percentage of infective juveniles after hours					Mean	Survival percentage of infective juveniles after hours					Mean
	24	48	72	96	120		24	48	72	96	120	
20	94.33 (76.31)	90.18 (71.76)	84.39 (66.74)	80.17 (63.58)	73.79 (59.21)	84.57 (66.89)	98.16 (82.29)	96.09 (78.61)	90.49 (72.05)	87.13 (68.95)	86.45 (68.44)	91.66 (73.26)
25	100.00 (88.19)	100.00 (88.19)	96.49 (79.22)	92.95 (74.66)	89.66 (71.28)	95.82 (78.17)	100.00 (88.19)	93.17 (74.88)	86.35 (68.36)	84.19 (66.58)	80.73 (63.94)	88.89 (70.54)
30	90.82 (72.34)	85.65 (67.78)	76.78 (61.21)	70.14 (56.85)	61.93 (51.88)	77.06 (77.06)	91.09 (72.64)	83.17 (65.80)	78.46 (62.38)	72.91 (58.63)	64.13 (53.19)	77.95 (62.03)
35	60.89 (51.30)	53.58 (47.06)	41.11 (39.87)	23.97 (29.33)	6.16 (14.42)	37.14 (37.52)	63.47 (52.83)	56.34 (48.62)	43.63 (41.32)	27.17 (31.44)	10.91 (19.28)	40.30 (39.41)
Mean	86.51 (68.44)	82.50 (65.27)	74.69 (59.80)	66.81 (54.82)	57.89 (49.55)		88.18 (69.91)	82.19 (65.05)	74.73 (59.80)	67.85 (55.49)	60.56 (51.12)	

Values in parenthesis are angular transformed values.

	F	SEM <u>+</u>	CD	F	SEM <u>+</u>	CD
Temperature	* *	1.0051	2.8714	* *	0.4933	1.4093
Time after hours	* *	1.1238	3.2103	* *	0.5516	1.5756
Temperature X Time	* *	2.2476	6.4206	* *	1.1031	3.1512

**Table 10 : Effect of Relative Humidity on Pathogenicity of entomopathogenic nematodes.**

RH level	% mortality of <i>G.mellonella</i> by <i>S.carpocapsae</i> after hours				Mean	% mortality of <i>G.mellonella</i> by <i>H.indica</i> after hours				Mean
	24	48	72	96		24	48	72	96	
50	58.12 (49.66)	50.39 (45.23)	36.13 (36.93)	22.13 (28.04)	41.69 (40.22)	62.39 (52.18)	54.86 (47.81)	41.25 (39.99)	35.34 (36.45)	48.46 (44.14)
60	61.96 (51.94)	54.17 (47.41)	39.96 (39.23)	30.27 (33.40)	46.59 (43.05)	66.43 (54.57)	59.36 (50.42)	47.73 (43.68)	40.53 (39.52)	53.51 (47.01)
70	73.15 (58.82)	69.26 (56.35)	60.73 (51.18)	56.94 (48.97)	65.02 (53.73)	80.73 (63.94)	73.51 (59.02)	64.16 (53.25)	60.74 (51.18)	69.79 (56.66)
80	88.16 (69.91)	81.39 (64.45)	76.94 (60.94)	66.43 (54.57)	78.10 (62.10)	92.16 (73.78)	83.42 (65.96)	79.17 (62.87)	71.56 (57.80)	81.58 (64.60)
Mean	70.35 (57.04)	63.80 (53.01)	53.31 (46.89)	43.94 (41.50)		75.43 (60.27)	67.79 (55.43)	58.08 (49.66)	52.04 (46.15)	

Values in parenthesis are angular transformed values.

	F	SEM	CD	F	SEM	CD
RH	* *	0.7465	2.1535	* *	0.5635	1.6258
Time after days	* *	0.6465	1.8650	* *	0.4880	1.4080
RH X Time	* *	1.4929	4.3071	* *	1.1271	3.2517

**Table - 15 : Effect of soil moisture on survival of entamopathogenic nematodes**

Soil moisture (%)	% survival of <i>S.carpocapsae</i> infective juveniles after hours					Mean	% survival of <i>H.indica</i> infective juveniles after hours					Mean
	24	48	72	96	120		24	48	72	96	120	
5	72.13 (58.12)	63.47 (52.83)	53.49 (47.01)	44.31 (41.73)	30.17 (33.34)	52.71 (46.55)	76.17 (60.80)	68.93 (56.10)	56.47 (48.73)	49.32 (44.60)	38.18 (38.17)	57.81 (49.49)
10	76.43 (60.94)	70.37 (57.04)	66.39 (54.57)	59.27 (50.36)	47.49 ( )	63.99 (53.13)	80.23 (63.58)	75.47 (60.33)	70.31 (56.98)	64.19 (53.25)	53.42 (46.95)	68.72 (55.98)
15	96.45 (79.22)	84.13 (66.50)	78.09 (62.10)	70.13 (56.85)	64.37 (53.37)	78.62 (62.44)	97.13 (80.20)	88.72 (70.36)	79.43 (63.01)	70.32 (56.98)	66.47 (54.63)	80.41 (63.72)
20	60.43 (51.00)	50.13 (45.06)	45.13 (42.19)	40.37 (39.47)	30.17 (33.34)	45.25 (42.30)	72.17 (58.18)	63.13 (52.59)	53.43 (46.95)	43.95 (41.55)	34.13 (35.73)	53.36 (46.95)
25	56.19 (48.56)	46.39 (42.94)	40.49 (39.52)	30.23 (33.34)	24.59 (29.73)	39.58 (39.00)	58.13 (49.66)	49.42 (44.66)	43.47 (41.27)	36.13 (36.93)	31.45 (34.14)	43.72 (40.80)
Mean	72.33 (58.24)	62.90 (52.48)	56.72 (48.85)	48.86 (44.37)	39.36 (38.88)		76.77 (61.21)	69.13 (56.23)	60.62 (51.12)	52.78 (46.66)	44.73 (41.96)	

Values in parenthesis are angular transformed values.

	F	SEM	CD	F	SEM	CD
Moisture	* *	0.4737	1.3598	* *	0.3793	1.0890
Time after hours	* *	0.4737	1.3598	* *	0.3793	1.0890
Moisture X Time	* *	1.0591	3.0406	* *	0.8482	2.4350

**Table - 16 : Effect of soil moisture on pathogenecity of entomopathogenic nematodes.**

Soil moisture (%)	% mortality of <i>G.mellonella</i> by <i>S.carpocapsae</i> after hours				Mean	% mortality of <i>G.mellonella</i> by <i>H.indica</i> after hours				Mean
	24	48	72	96		24	48	72	96	
5	53.43 (46.95)	43.12 (41.03)	30.18 (33.34)	23.92 (29.27)	37.66 (37.88)	60.69 (51.18)	49.73 (44.83)	40.16 (39.35)	30.37 (33.46)	45.24 (42.25)
10	65.73 (54.15)	55.66 (48.27)	48.13 (43.91)	39.13 (38.70)	52.16 (46.26)	69.13 (56.23)	61.76 (51.83)	56.34 (48.62)	43.13 (41.03)	57.59 (49.31)
15	86.97 (68.87)	80.13 (63.51)	72.47 (58.37)	64.32 (53.31)	75.97 (60.67)	91.13 (72.64)	82.36 (65.20)	76.15 (60.80)	70.34 (56.98)	79.99 (63.43)
20	60.13 (50.83)	51.43 (45.80)	43.15 (41.09)	32.96 (35.06)	46.92 (43.22)	64.96 (53.73)	52.16 (46.26)	46.27 (42.88)	39.74 (39.06)	50.78 (45.46)
25	50.16 (45.11)	41.43 (40.05)	31.17 (33.96)	20.76 (27.13)	35.88 (36.81)	56.13 (48.50)	49.42 (44.66)	38.93 (38.59)	30.64 (33.58)	43.78 (41.44)
Mean	63.28 (52.71)	54.35 (47.52)	45.02 (42.13)	36.22 (36.99)		68.41 (55.80)	59.09 (50.24)	51.57 (45.92)	42.84 (40.86)	

Values in parenthesis are angular transformed values.

	F	SEM	CD	F	SEM	CD
Soil moisture	* *	0.4737	1.3598	* *	0.3793	1.0890
Time after hours	* *	0.4466	1.2757	* *	0.5290	1.5112
Moisture X Time	* *	0.9986	2.8526	* *	1.1828	3.3790

**Table 14 : Effect of p<sup>H</sup> on Pathogenicity of entomopathogenic nematodes.**

p <sup>H</sup>	% mortality of <i>G.mellonella</i> by <i>S.carpocapsae</i> after hours				Mean	% mortality of <i>G.mellonella</i> by <i>H.indica</i> after hours				Mean
	24	48	72	96		24	48	72	96	
5	65.37 (53.97)	50.36 (45.23)	45.38 (42.36)	36.63 (37.23)	49.44 (44.66)	76.19 (60.80)	56.37 (48.68)	50.13 (45.06)	40.67 (39.64)	55.84 (48.33)
6	75.37 (60.27)	60.39 (51.00)	53.42 (46.95)	45.37 (42.36)	58.64 (49.95)	88.43 (70.09)	65.49 (54.03)	57.13 (49.08)	49.09 (44.48)	65.04 (53.73)
7	96.43 (79.06)	86.43 (63.72)	73.13 (58.76)	64.42 (53.37)	78.60 (62.44)	98.19 (82.29)	82.37 (65.20)	77.49 (61.68)	70.18 (56.91)	82.06 (64.97)
8	78.12 (62.10)	50.43 (45.23)	44.31 (41.73)	38.47 (38.35)	52.83 (46.61)	82.13 (64.97)	55.49 (48.16)	47.63 (43.62)	43.42 (41.21)	57.17 (49.14)
9	60.32 (50.94)	45.89 (42.65)	33.49 (35.37)	21.37 (27.56)	40.27 (39.41)	64.39 (53.37)	51.37 (45.80)	49.49 (44.71)	28.17 (32.08)	48.34 (44.03)
Mean	75.12 (60.07)	57.50 (49.31)	49.95 (45.0)	41.25 (39.99)		81.87 (64.82)	62.22 (52.06)	56.37 (48.68)	46.31 (42.88)	

Values in parenthesis are angular transformed values.

	F	SEM <sub>±</sub>	CD	F	SEM <sub>±</sub>	CD
pH	* *	0.8416	2.4043	* *	0.7367	2.1046
Time after hours	* *	0.7528	2.1505	* *	0.6589	1.8824
pH X Time	* *	1.6833	4.8086	* *	1.4734	4.2092

**Table - 24 : Pathogenicity of entomopathogenic nematodes on Lepidopteran pests**

Nematode Inoculum level (IJs / larvae)	% mean mortality of larvae					
	<i>Helicoverpa armigera</i>		<i>Spodoptera litura</i>		<i>Papilio demoleus</i>	
	<i>S.carpocapsae</i>	<i>H.indica</i>	<i>S.carpocapsae</i>	<i>H.indica</i>	<i>S.carpocapsae</i>	<i>H.indica</i>
20	20.83 (27.13)	22.53 (28.32)	23.95 (29.33)	26.65 (31.11)	27.65 (31.82)	28.55 (32.33)
40	39.90 (39.17)	40.65 (39.64)	40.65 (39.64)	41.95 (40.40)	41.95 (40.40)	43.30 (41.15)
60	55.25 (48.04)	57.43 (49.26)	46.75 (43.17)	49.93 (44.94)	75.73 (60.47)	76.45 (61.00)
80	66.65 (54.76)	67.95 (55.55)	54.93 (47.81)	56.95 (49.02)	74.83 (59.87)	80.63 (63.87)
Control	0.00	0.00	0.00	0.00	0.00	0.00

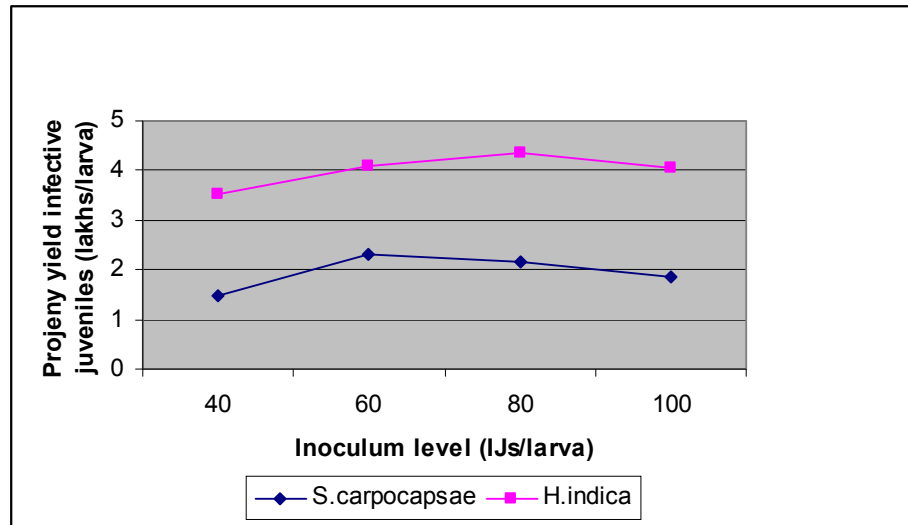
Values in parenthesis are angular transformed values.

**Table - 25 : Median Lethal Dosage (LD<sub>50</sub>) and Median Lethal Time (LT<sub>50</sub>) of entomopathogenic nematodes on Three Lepidopteran Pests.**

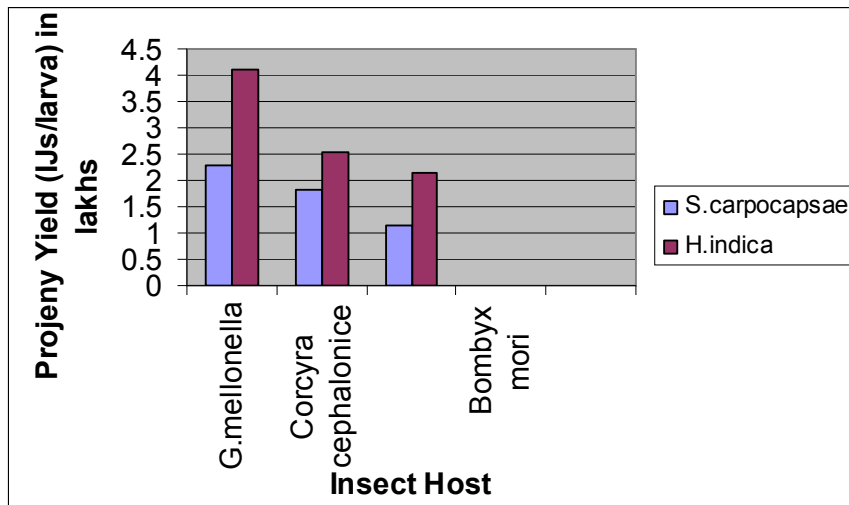
LD <sub>50</sub>							LT <sub>50</sub>						
Time (hrs)	Nematode inoculum (Ijs / larvae)						Inoculum level (IJs)	Time (hours)					
	<i>H.armigera</i>		<i>S.litura</i>		<i>P.demoleus</i>			<i>H.armigera</i>		<i>S.litura</i>		<i>P.demoleus</i>	
	<i>S.carpocapsae</i>	<i>H.indica</i>	<i>S.c</i>	<i>H.i</i>	<i>S.c</i>	<i>H.i</i>		<i>S.c</i>	<i>H.i</i>	<i>S.c</i>	<i>H.i</i>	<i>S.c</i>	<i>H.i</i>
24	69.31	58.60	95.50	140.3	72.11	59.31	20	154.10	140.30	151.8	170.2	138.8	129.5
48	58.20	51.40	65.16	59.02	41.69	41.69	40	91.41	92.40	71.61	94.15	73.45	76.59
72	46.99	44.77	45.50	43.15	33.19	33.88	60	33.34	33.50	54.08	55.21	44.46	20.30
96	35.24	37.30	28.67	28.89	26.55	26.12	80	23.55	21.58	42.95	31.05	21.63	11.43

Mass multiplication of EPNs on *G.mellonella*  
 Inoculum level (IJs/larva)  
 Progeny yield infective juveniles (lakhs/larva)

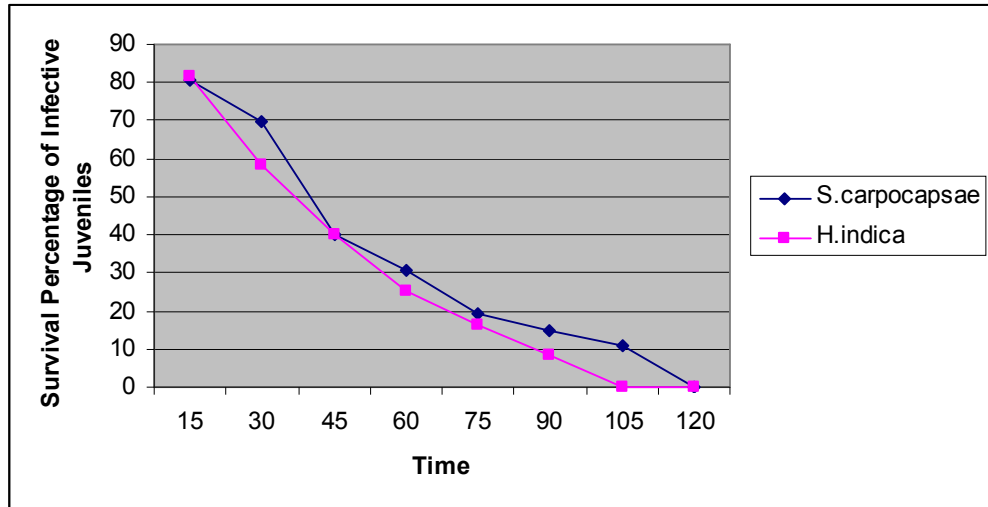
**Fig 1 : Mass multiplication of EPNs on *G.mellonella***



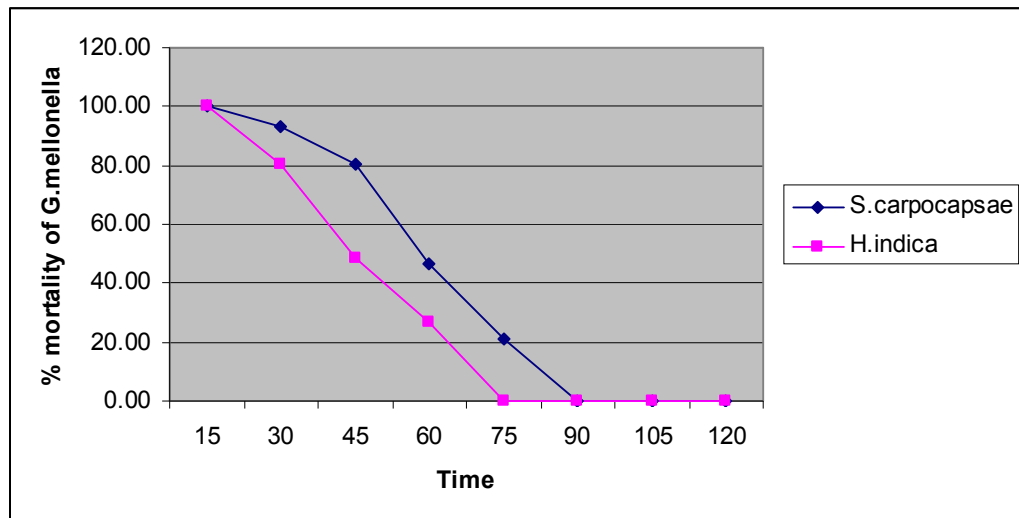
**Fig 2 : Progeny production of EPN isolates from different insect hosts**



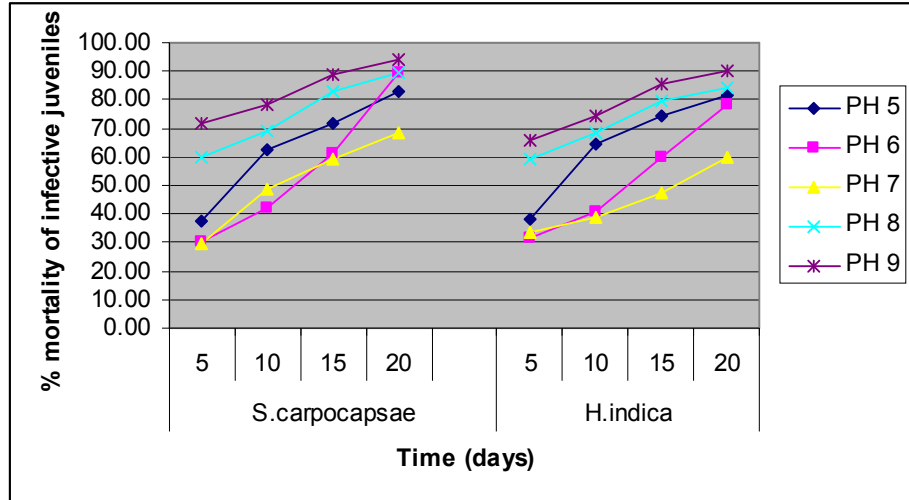
**Fig 7 : Effect of U.V.Radiation on the survival of entomopathogenic nematodes**



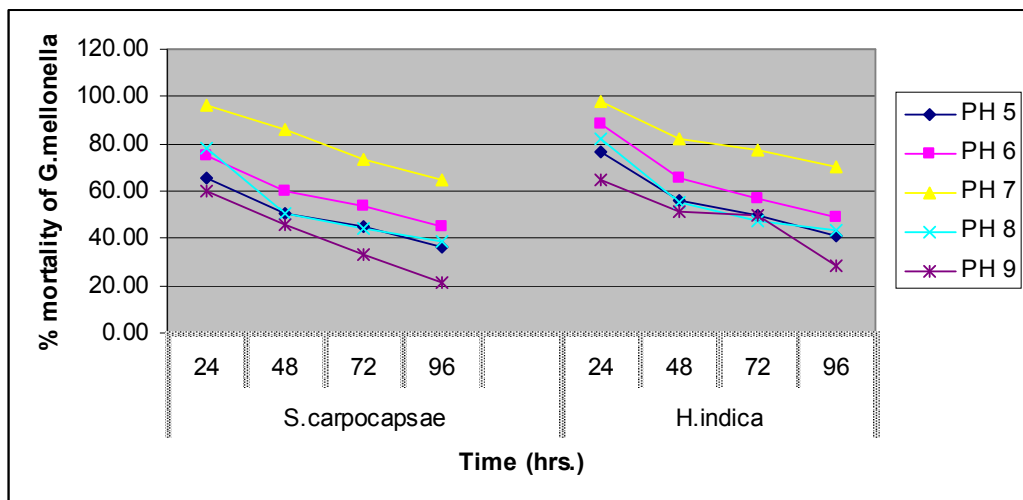
**Fig.8 : Effect of U.V.Radiation on Pathogenicity of entomopathogenic nematodes**



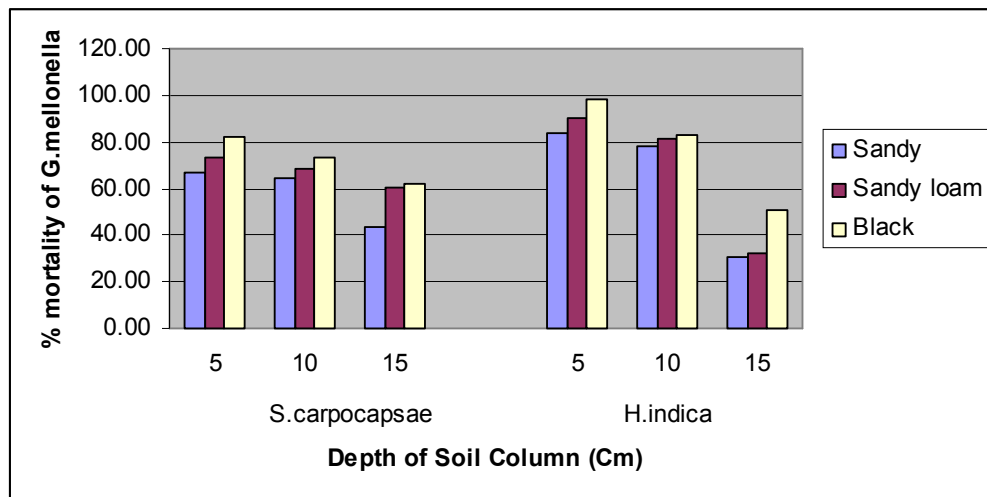
**Fig 9 : Effect of p<sup>H</sup> on mortality / survival of entomopathogenic nematodes**



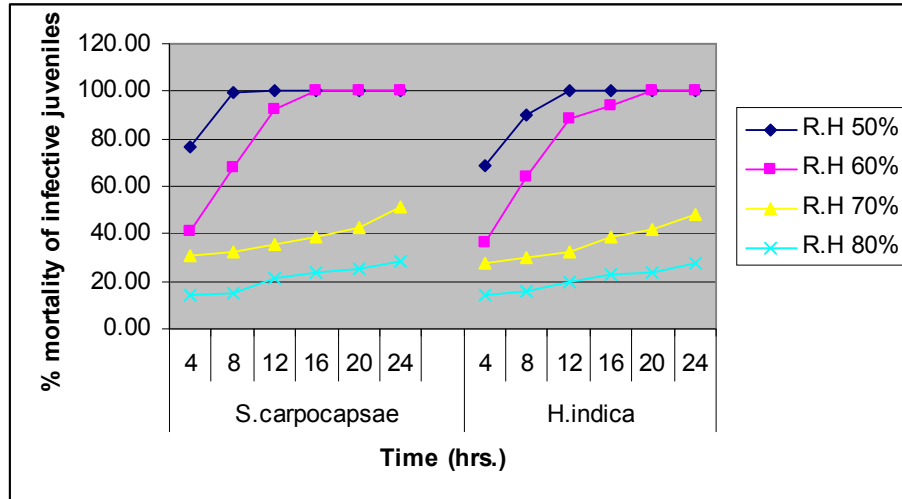
**Fig 10 : Effect of p<sup>H</sup> on Pathogenicity of entomopathogenic nematodes**



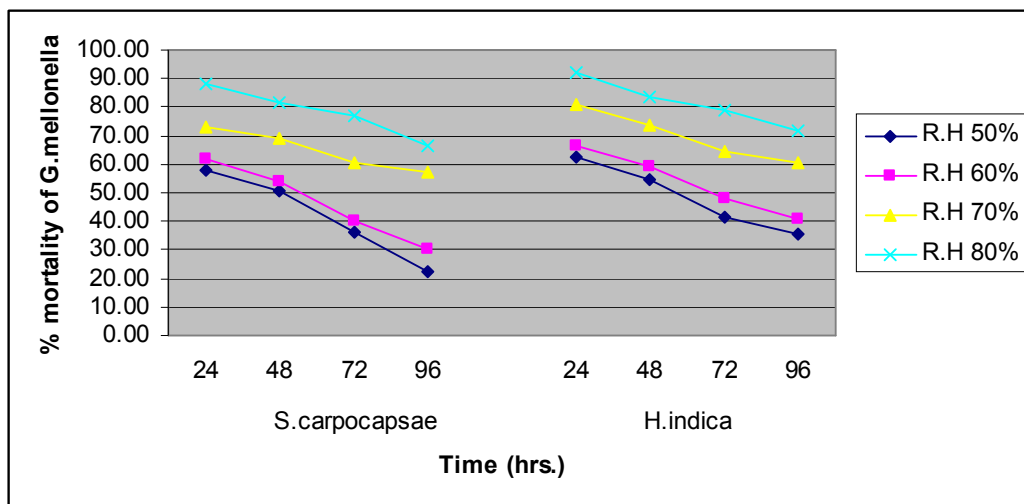
**Fig 13 : Effect of soil type on pathogenicity of entomopathogenic nematodes**



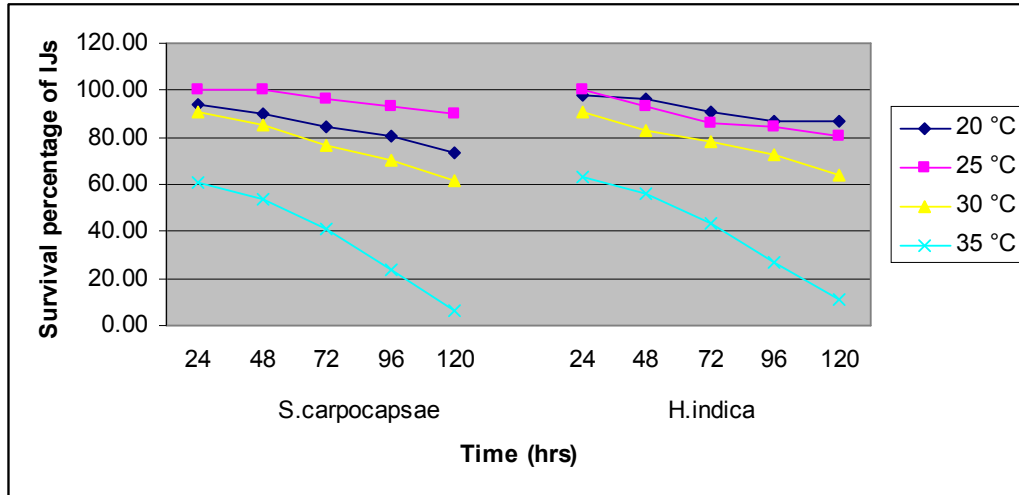
**Fig 5 : Effect of Relative Humidity on mortality / survival of entomopathogenic nematodes**



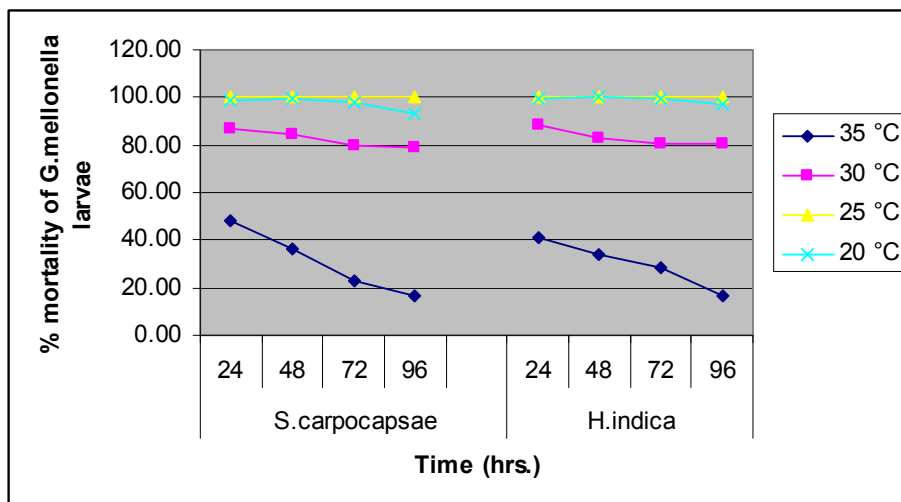
**Fig 6 : Effect of Relative Humidity on Pathogenicity of entomopathogenic nematodes**



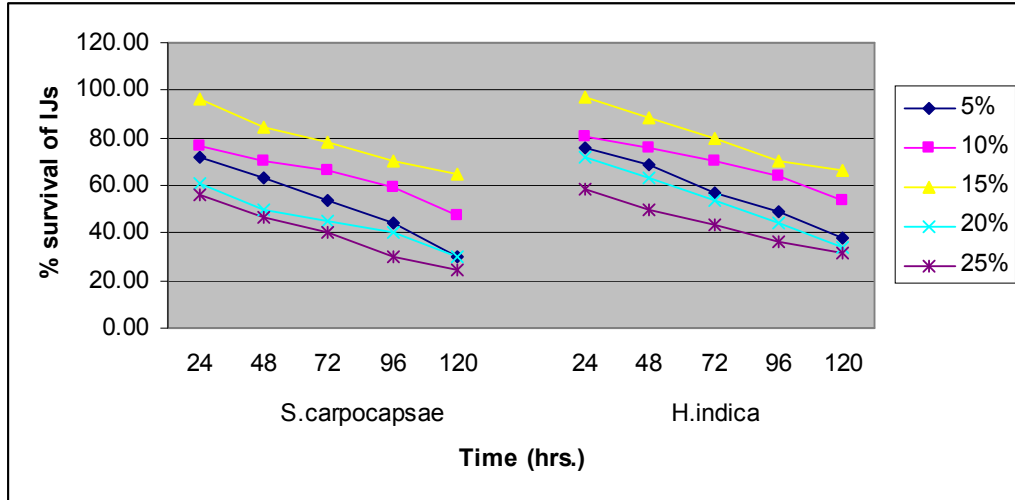
**Fig 3 : Effect of temperature on survival of entomopathogenic nematodes**



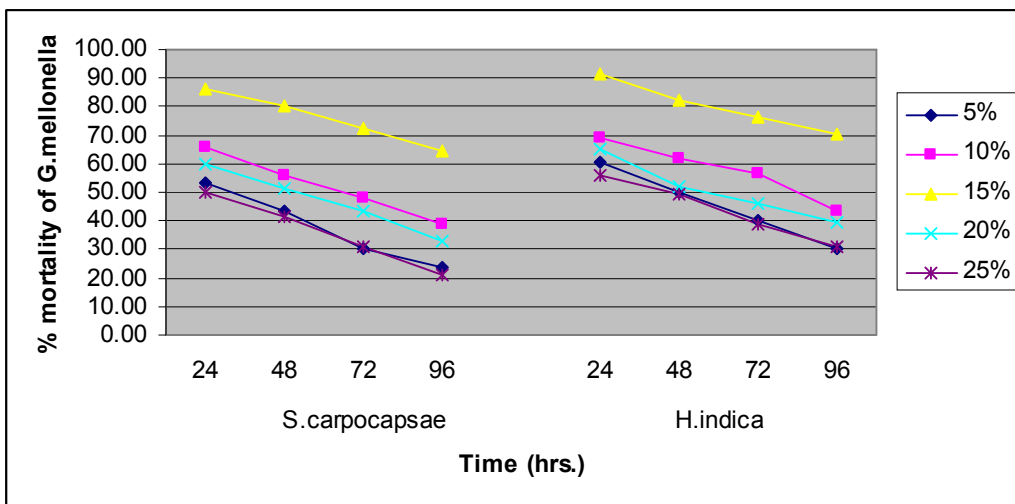
**Fig 4 : Effect of temperature on pathogenicity of entomopathogenic nematodes**



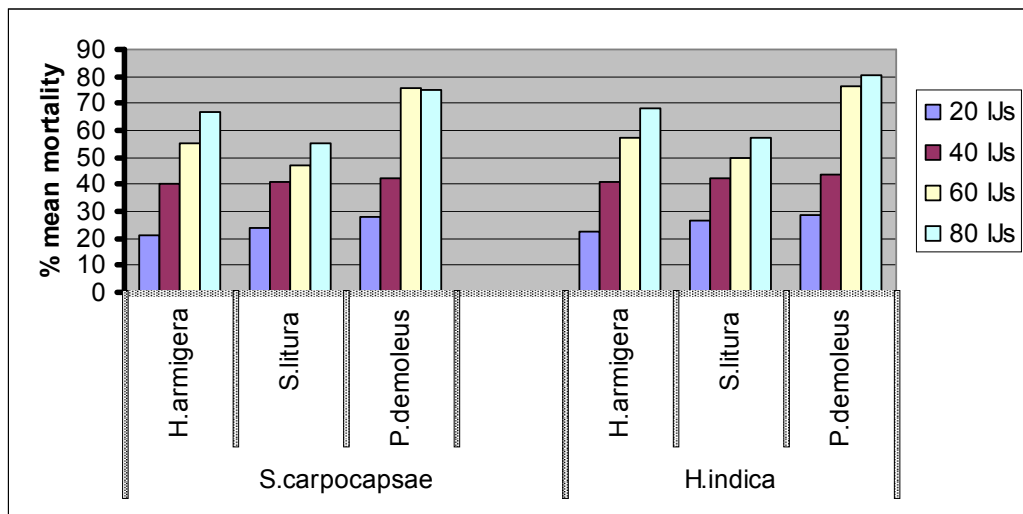
**Fig 11 : Effect of soil moisture on survival of entomopathogenic nematodes**



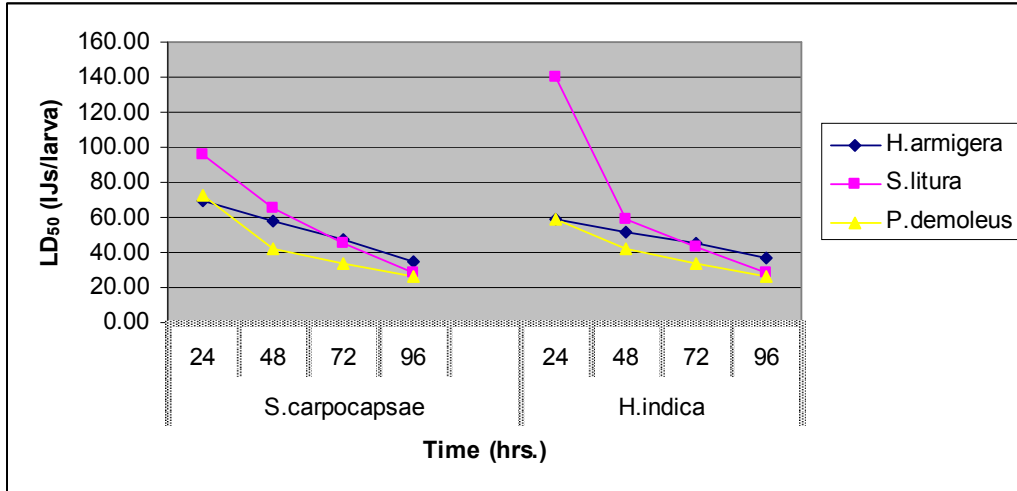
**Fig 12 : Effect of soil moisture on pathogenicity of entomopathogenic nematodes**



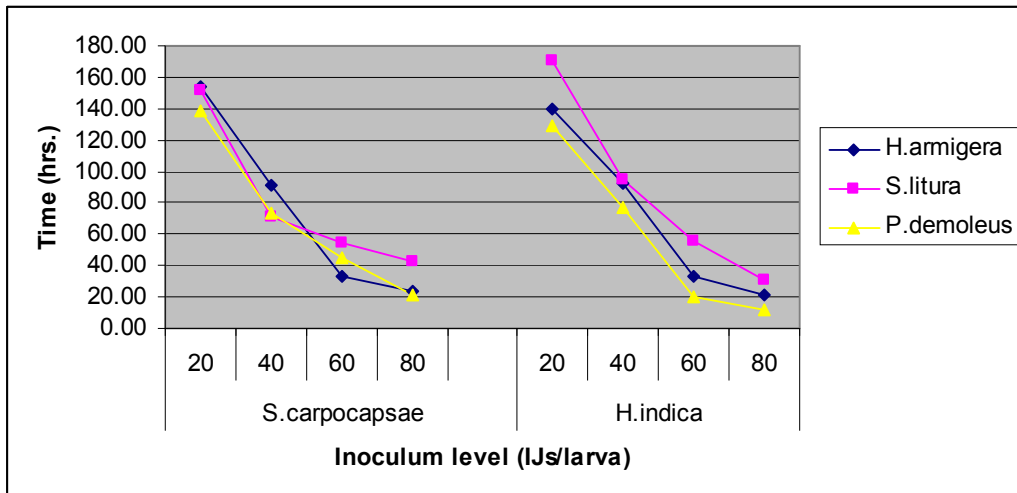
**Fig 14 : Pathogenicity of entomopathogenic nematodes on Lepidopteron pests**



**Fig 15 : Median Lethal Dosage (LD<sub>50</sub>) of entomopathogenic nematodes on three Lepidopteran pests**



**Fig 16 : Median Lethal Time (LT<sub>50</sub>) for entomopathogenic nematodes on three Lepidopteran pests**







## **CHAPTER - V**

### **DISCUSSION**

The results documented through various studies i.e. survey for isolation of entomopathogenic nematodes, identification and mass multiplication, influence of environmental factors on disease infection by entomopathogenic nematodes and evaluation of EPNs for their pathogenicity against lepidopteran pests are discussed here under different headings.

#### **5.1 SURVEY FOR ISOLATION OF ENTOMOPATHOGENIC NEMATODES**

A total of 135 samples were collected during January - June, 2005 for isolation of entomopathogenic nematodes. Results revealed the absence of nematodes in any of the soil samples, which might be due to the prevalence of high atmospheric temperature, low relative humidity and long drought spell for the past two years, resulting in the suppression of growth and development of the entomopathogenic nematodes in the soil. These results were further confirmed by Gaulger (1981) who has emphasized that infective juveniles of entomopathogenic

nematodes are very sensitive to environmental extremes such as temperature and relative humidity. It was reported that optimum level of temperature for development of infective juveniles was 25°C and no development and reproduction of infective juveniles could be observed when the atmosphere temperature goes more than 33°C and relative humidity was below 50 per cent. However, it is contrary to the results of Hussaini (2001), Singh *et al.* (1992) and Prasad and Katti (2003) who recovered different isolates of entomopathogenic nematodes by collecting the soil samples, when the atmosphere temperature was 20 to 25°C, which was considered as the optimum temperature for nematode development.

### **5.2.1 Identification and mass multiplication of entomopathogenic nematodes**

Mass multiplication of entomopathogenic nematodes was carried out on *G. mellonella* larva by inoculating different levels of infective juveniles i.e. 40, 60, 80 and 100. Results revealed that *G. mellonella* yielded maximum of 2.3 lakhs progeny when 60 infective juveniles per larva were inoculated, lowest of 1.49 lakh progeny when 40 infective juveniles per larva were inoculated.

Whereas in the case of *H. indica* maximum progeny of 4.34 lakhs was recorded at an inoculum level of 80 infective juveniles per larva and lowest progeny of 3.52 lakhs at 40 infective juveniles level. The difference in inoculum level of infective juveniles per larva from *G. mellonella* ultimately resulted in a high progeny production of *H. indica* than *S. carpocapsae*. This might be due to the reason that a single *G. mellonella* larva could accommodate more number of infective juveniles that yielded maximum of 4.34 lakhs at infective juveniles of *H. indica* as compared to *S. carpocapsae* where a single larva of *G. mellonella* yielded only 2.3 lakh infective juveniles. This is due to the reason that a single *G. mellonella* larva could not provide required food material for 80 IJS of *S. carpocapsae* as compared to *H. indica*. These results are similar to those reported by P.D.B.C (2002) where they have obtained highest infective juveniles ranging from *G. mellonella* by inoculating infective juveniles ranging from 50 to 75 and they have recorded highest yield of *S. carpocapsae* at 50 IJS inoculum level and of *H. bacteriophora* at 75 IJS inoculum level.

### **5.2.2 Yield of entomopathogenic nematodes from larvae of different sizes of greater wax moth, *G. mellonella***

Results regarding yield of progeny of the infective juveniles from the larvae of *G. mellonella* varying in size and weight revealed that as the weight of the larva was increased, progeny that could be produced from the larva also increased. more progeny production was recorded in the case of *H. indica* compared to *S. carpocapsae* i.e. 2,01,560 infective juveniles of *H. indica* were recorded from large sized larvae (with 20-22 mm body length and weight to 340 mg), whereas *S. carpocapsae* could yield only 1,02,400 juveniles from the same sized larvae (20-22 mm body length, 350 mg weight). Small sized larvae (12-14 mm body length and weight of 140 mg) yielded about 1,06,000 infective juveniles of *H.india* and almost same sized larvae of 10-12m.m bodylength and weight of 150mg, yielded 81,340 infective juveniles of *S.carpocapsae*. The difference in number of IJS production among the two EPNs i.e. *S.carpocapsae* and *H.indca* might be due to large size of the larva the could provide sufficient nourishment for the developing infective juveniles as compared to small sized larvae. These results confirm the results of Rajkumar *et al.* (2002) who have obtained high progeny yield of *H. indica* from large sized larva (size of 20 to 22 mm, weight of 223 to 257 mg) than *S. carpocapsae*.

### **5.2.3 Progeny production of EPN isolates from different insect hosts**

When three different lepidopteran insect larvae i.e. *Galleria mellonella*, *Corcyra cephalonica* and *Bombyx mori* were tested for the efficacy in the production of two nematode species i.e. *S. carpocapsae* and *H. indica*, it was clear that among all the three test insects, *G. mellonella* recorded highest yield of *H. indica* (4.1 lakhs) and *S. carpocapsae* (2.3 lakhs) followed by *C.cephalonica* (2.52 lakhs of *H. indica* and 1.83 lakhs of *S. carpocapsae*) and *B. mori* (2.13 lakhs *H. indica* and 1.13 lakhs *S. carpocapsae*). The present results are similar to those obtained by Hussaini *et al.*, (1998) where they recorded highest yield of infective juveniles from the larvae of *G. mellonella* followed by *Agrotis ipsilon*, *Cocyrta cephalonica*, *Spodoptera litura*, *Helicoverpa armigera*. These results are also in confirmation with the findings of Zaki *et al.* (2000) where they have observed low progeny yield from third instar larvae of *B. mori*, i.e 2750 IJS of *H. bacteriophora* and 48,703 IJS of *S. carpocapsae*. However, a slight increase in the progeny yield of *S. carpocapsae* and *H. indica* with *B. mori* in the present work when compared with the results of Zaki *et al.* (2000) might be due to the difference in the instar stage of larvae used for multiplication of the entomopathogenic nematodes.

### **5.3 EFFECT OF ENVIRONMENTAL FACTORS ON NEMATODE SURVIVAL AND PATHOGENICITY**

#### **5.3.1 Effect of temperature on survival of entomopathogenic nematodes**

Present studies on the effect of temperature on nematode survival revealed that 25°C was the optimum temperature for maximum survival i.e. 89.16 per cent of the infective juveniles of the *S. carpocapsae*. Temperature of 35°C was found to be unfavourable showing only 37.14 per cent survival. In the case of *H. indica*, a maximum of 91.66 per cent survival of the juveniles was observed at 20°C and lowest survival of 40.3 per cent at 35°C was recorded. Present results are similar to the findings of Danilov, 1976 and Pye and Burman, 1978 who have reported, 25°C as the optimum temperature for growth and development of Steinernematids and with special reference to *S. carpocapsae*. However, the present observation coincide with those of Ghally (1995) who has given 20-25°C was the suitable temperature for the activity of *H. heliothidis* and *S. carpocapsae*.

### **5.3.1.1 Effect of temperature on pathogenicity of entomopathogenic nematodes**

With regard to the nematode pathogenicity against the larvae of *G. mellonella*, it was clear that *S. carpocapsae* and *H. indica* recorded 100 per cent larval mortality at 25°C followed by 20°C and 30°C. Whereas at 35°C, minimum larval mortality of 31.05 per cent by *S. carpocapsae* and 30.03 per cent by *H. indica* was recorded, following the results of Danilov (1976) who have observed maximum larval mortality at temperatures ranging from 10-32°C, and Molyneux (1986) according whom the acceptable temperature for nematode survival, development and infection for disease development by *S. carpocapsae* was 10-28°C. Present results confirm with the statement of Shang Ping King and Randy Gaugler (1991) that lower temperature of 25°C favour the survival and pathogenicity of *S. carpocapsae* than higher temperature of 35°C.

### **5.3.2 Effect of relative humidity on mortality / survival of entomopathogenic nematodes**

Results obtained from the experiment on effect of relative humidity on nematode survival or mortality revealed that a maximum survival of 79.92 per cent of the infective juveniles of *S. carpocapsae* and 79.5 per cent of *H. indica* was recorded at 80.00 per cent relative humidity. At 50 per cent relative humidity, low survival rate of 4.17 per cent of *S. carpocapsae* and 7.0 per cent of *H. indica* was recorded. These results are similar to the findings of Simons and Poinar (1973), who observed more survival of *S. carpocapsae* at 85 per cent relative humidity. These results further confirm with the results of Kung *et al.* (1991) who observed increasing survival of the IJS of *S. feltiae* and *S. glaseri* with increasing relative humidity from 25 to 100 per cent. Present results are also similar to the findings of Kamionek *et al.* (1974) who observed more survival of *S. carpocapsae* at 85 per cent humidity level. More survival of the infective juveniles at high humidity levels might be due to absence of desiccation from the body of the nematodes as compared to low humidity level.

### **5.3.2.1 Effect of relative humidity on pathogenicity of entomopathogenic nematodes**

Results pertaining to the effect of relative humidity on nematode pathogenicity against *G. mellonella* showed that pathogenicity of *S. carpocapsae* and *H. indica* decreased as the relative humidity decreased. There was a linear relationship between pathogenicity of the test insect and relative humidity. *H. indica* showed maximum mortality of 81.58 per cent of the *G.mellonella* larvae at 80 per cent relative humidity level whereas only 78 per cent with *S. carpocapsae* at same level of relative humidity. However, at 50 per cent humidity level, *H. indica* showed a maximum of 48.56 per cent pathogenicity of the test insect and *S. carpocapsae* recorded only 41.69 per cent mortality of *G. mellonella* showing that *S. carpocapsae* was more sensitive to low humidity levels (50%) compared to *H. indica*. These results further confirm the results of Moore (1965) where they who observed increase in pathogenicity of *S. carpocapsae* with increasing level of relative humidity. These results also are similar to the findings of King *et al.* (1991) where they have recorded a decrease in pathogenicity by the two nematode species i.e. *S.carpocapsae* and *S. glaseri* with decreasing relative humidity from 100 to 25 per cent.

### **5.3.3 Effect of U.V radiation on survival of entomopathogenic nematodes**

In the present study, results pertaining to the effect of U.V. radiation on nematode survival revealed that, in case of *S. carpocapsae* absolute mortality was recorded after 120 minutes of exposure to U.V. radiation at 254 nm wave length, whereas for *H. indica* mortality was recorded within 105 minutes. It was clear from the present study that with increasing period of exposure to UV radiation survival of infective juveniles of both *S. carpocapsae* and *H. indica* was reduced recording high mortality of the juveniles. Similar observations were made by Gaugler and Boush (1978) and Gulsar Banu and Rajendran (2003) who demonstrated that inactivation of juveniles of *S. carpocapsae*, occurred after irradiation with a germicidal lamp emitting peak wave length of 254 nm. They have also recorded absolute mortality of the infective juveniles of *H. indica*, *S. glaseri* and *Steinernema species* after exposing to U.V. light for a period of 120-135 minutes. However, the results were contrary to the results of Cheng – Chichin, (1997), who reported that all nematodes were dead after continuous exposure to U.V. light for 36 hrs. This variation in

survival of infective juveniles might be due to different periods of exposure to U.V. light as well as difference in wave lengths of the U.V. light used.

#### **5.3.3.1 Effect of U.V. radiation on pathogenicity of entomopathogenic nematodes**

Experiment on the effect of U.V. radiation on nematode pathogenicity against *G. mellonella* revealed that per cent infectivity of irradiated juveniles of both *S. carpocapsae* and *H. indica* against *G. mellonella* was decreased with increasing exposure period to U.V. light. Complete loss of infectivity of juveniles of *H. indica* was observed after 60 minutes of exposure period whereas in case of *S. carpocapsae*, it was after 75 minutes. Hence, it was concluded that *H. indica* was more sensitive to U.V. radiation than *S. carpocapsae*. These results are similar to the results of Gulsar Banu and Rajendran (2003) where they have observed decrease in infectivity of infective juveniles with increasing exposure period to U.V. radiation. In the present study initial loss of infectivity was observed immediately after 15 minutes for both *S. carpocapsae* and *H. indica*. These results are contrary to Gaugler and Boush (1978) where they have observed

the loss of pathogenicity in *S. feltiae* within 7 minutes and Randy Gaugler *et al.* (1992) who reported the same in *H. bacteriophora* at 4 minutes of irradiation and after 6 minutes for *S. carpocapsae*. The difference in pathogenicity of the infective juveniles of either *S. carpocapsae* or *H. indica* at different time intervals might be due to the species variation as well as wave length of light used in the experiment.

#### **5.3.4 Effect of pH on mortality / survival of entomopathogenic nematodes**

Results pertaining to the effect of pH on mortality or survival of the infective juveniles of the two nematode species i.e. *S. carpocapsae* and *H. indica* revealed that at pH 7, maximum survival of nematodes i.e. 46.05 per cent of *S. carpocapsae* and 55.12 per cent of *H. indica* was observed. With increase in pH to 9, survival was decreased to 16.80 per cent in the case of *S. carpocapsae* and 21.03 per cent in the case of *H. indica*. When the pH was decreased to 5, the survival of the infective juveniles was decreased to 36.42 per cent in *S. carpocapsae* and 33.88 per cent in *H. indica*. Similar results were reported by Kung *et al.* (1990b) who observed lowest survival of the infective juveniles of

the two nematode species *i.e.* *S. carpocapsae* and *S. glaseri* at acidic pH of 5 and basic pH of 10 and confirm the reports made by Cheng – Chichin (1997) who observed low nematode survival under high acidic conditions.

#### **5.3.4.1 Effect of pH on pathogenicity of entomopathogenic nematodes**

Experiment conducted to know the influence of pH on nematode pathogenicity against *G. mellonella* recorded high larval mortality of 82.06 per cent at a pH of 7. Whereas at acidic level *i.e.* pH of 5, low mortality of 55.84 per cent was observed. When the pH was raised to 9 towards alkaline range, there was a slight decrease in mortality upto 48.34 per cent. Present results are similar to those observed by Fischer and Fuhver, 1990 according to whom, under acidic conditions *i.e.* pH remaining below four movement of infective juveniles of *S. kraussei* was limited for finding the host. These results confirm the reports of Kung *et al.* (1990b) according to whom it was clear that at all pH levels, infective juveniles of both *S. carpocapsae* and *H. indica* could be able to infect *G. mellonella* larvae and cause mortality either low or highest.

Finally it could be concluded that slightly acidic or alkaline conditions also allow nematodes to survive and cause disease in insect host larvae.

### **5.3.5 Effect of soil moisture on survival of entomopathogenic nematodes**

Observations pertaining to the effect of soil moisture on survival of the infective juveniles of the two species i.e. *S. carpocapsae* and *H. indica* revealed that juveniles of both the nematode species showed maximum survival at a moisture level of 15 per cent i.e. 78.62 per cent in the case of *S. carpocapsae* and 80.41 per cent in the case of *H. indica*. When the moisture level was raised to 25 per cent, there was a sharp decrease in survival of the infective juveniles i.e. 39.58 per cent of *S. carpocapsae* and 43.72 per cent of *H. indica*. At almost 5 per cent moisture level, a decrease in survival of juveniles i.e. 52.71 per cent of *S. carpocapsae* and 57.81 per cent of *H. indica* was observed. This difference in rate of survival might be due to the reason that at 15 per cent moisture level, sufficient aeration was there for nematode activity and survival. Whereas at 25 per cent moisture level, the excess soil moisture might have altered the soil aeration

and further affecting the nematode survival. The high rate of mortality of the juveniles of the two species, *S.carpocapsae* and *H.indica* at 5 per cent moisture level might be because of dessication due to insufficient moisture. These results are in conformation with the observations of Molyneux and Bedding (1984) who found decreased infectivity at low water potential ( $> -10$  bars) as well as at high water potential ( $<0.01$  bars) in sandy soils. However, present results are contrary to reports of Kung *et al.* (1990a) who reported that saturated condition of the soil was detrimental for survival of *S. carpocapsae* and *S. glaseri* and optimum survival could be at 2 to 4 per cent moisture level. The variation in the survival rate of infective juveniles of both *S. carpocapsae* and *H. indica* from the previous research work might be due to the variation in type of soil, influencing the moisture retention.

#### **5.3.5.1 Effect of soil moisture on pathogenicity of entomopathogenic nematodes**

Experiment conducted on effect of soil moisture on nematode pathogenicity against *G. mellonella* under laboratory conditions revealed that at 15 per cent moisture level, *S. carpocapsae* showed

a maximum larval mortality of 75.97 and *H. indica*, 79.99 per cent. When the moisture level was increased to 25 per cent, a decrease in larval mortality to 20.76 per cent by *S. carpocapsae* and 43.78 per cent by *H. indica* was observed. At a moisture level of 5.0 per cent mortality of 23.92 per cent by *S. carpocapsae* and 45.24 per cent by *H. indica* was recorded. This might be due to the fact that at field capacity level (15%) because of the optimum availability of oxygen, infective juveniles will be active and survive, so that they will not lose their pathogenicity against the *G. mellonella*. The results confirm the findings of Kung *et al.* (1991) who observed more infection in the larvae of *G. mellonella* due to *S. carpocapsae* and *S. glaseri* at 16.0 per cent moisture level. Similar results were obtained by Ghally (1995) who reported that increased activity of *H. heliothidis* and *S. carpocapsae* and their pathogenicity against cotton leaf worm was increased when the soil moisture level to field capacity.

#### **5.3.6 Effect of soil type on pathogenicity of entomopathogenic nematodes**

From the studies made on the effect of soil type on nematode pathogenicity against larvae of *G. mellonella*, it was clear that high

larval mortality of the test insect was observed in clayey soils by both *S. carpocapsae* and *H. indica*, showing 72.60 per cent by *S. carpocapsae* and 77.28 per cent by *H. indica*. This is followed by sandy loam soil, which showed 67.33 per cent and 67.86 per cent larval mortality by *S. carpocapsae* and *H. indica*. Very low mortality of 58.20 per cent by *S. carpocapsae* and 64.06 per cent by *H. indica* was recorded in sandy soil. The high larval mortality of *G. mellonella* in clayey soil might be due to the high clay content that favoured more moisture retention, ultimately creating suitable conditions for infection and disease development by infective juveniles of both the nematodes species. Low mortality of *G. mellonella* in sandy soil was due to rapid drying of the soil moisture that has ultimately resulted in causing low larval mortality. Similar results were made by Hominick and Briscoe (1990) according to who due to high organic matter content in black soil, nematodes showed more survival causing high larval mortality. Present results also confirm the findings of Kung *et al.* (1990a) who reported that infective juveniles of *S. carpocapsae* were active in sandy loam than in sandy soil which was confirmed in the present studies showing high larval mortality in sandy loam soil compared to sandy soil. In addition to these reasons it was emphasized by Barbercheck and Kaya (1991a) that the infective juveniles showed more mortality in sandy loams resulting in high

mortality of the larvae of *G. mellonella*. Present results are supported by the observations of Hussaini *et al.* (2000) who recorded maximum mortality of *Agrotis ipsilon* in sandy soil than loam soils.

Experiment conducted with regard to the influence of depth of soil on pathogenicity of *G. mellonella* by *S. carpocapsae* and *H. indica* recorded that as the depth of soil increased the pathogenicity decreased. At about 5 cm depth, mortality of larvae of *G. mellonella* was recorded as 77.33 by *S. carpocapsae* and 90.7 by *H. indica* followed by 10 cm depth showing 68.60 per cent by *S. carpocapsae* and 37.66 per cent *H. indica*. Similar results were made by Hussaini and Sankaranarayanan (2001), where maximum larval death of *G. mellonella* was recorded at 5 cm depth than at 10 cm depth. This might be due to the reason that as the soil depth, increased the distance for movement of infective juveniles of both nematodes i.e. *S. carpocapsae* and *H. indica* in reach of the host larvae increased hence larvae may be unable to cause disease resulting in low larval mortality at 15 and 10 cm depth levels compared to 5 cm depth of the soil.

## **5.4 EVALUATION OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF IMPORTANT LEPIDOPTERAN PEST**

### **5.4.1 Pathogenicity of *S. carpocapsae* / *H. indica* *Helicoverpa armigera***

Evaluation of entomopathogenic nematodes for the management of three important lepidopteran pests i.e. *Helicoverpa armigera*, *Spodoptera litura* and *Papilio demoleus* was carried out by inoculating infective juveniles of *S. carpocapsae* and *H. indica* with different inoculum levels of 20, 40, 60 and 80 IJS per larva under laboratory conditions. Results revealed that there was a linear relationship between dosage of nematodes and per cent mortality of the larvae of *H. armigera* by the two nematode species. Highest larval mortality of 83.40 per cent was recorded at 80 infective juvenile level. It was also calculated that the LD<sub>50</sub> value of *S. carpocapsae* for 50 per cent mortality of *H. armigera* was 35.24 IJS per larva and LT<sub>50</sub> as 23.35 hrs. In the case of *H. indica*, 84.40 per cent larval mortality was recorded at 80 infective juveniles. LD<sub>50</sub> of *H. indicia* for 50 per cent mortality of *H. armigera* was 21.58 hrs and LT<sub>50</sub> as 37.30 hrs was recorded. Present results coincide with the results of Tohirand Otto (1995), where there was linear relationship between dosage of nematodes and larval

morality. However, it is contrary to the observations of Glager and Navon (1990) where they recorded LD<sub>50</sub> for *S. carpocapsae* as 54 IJS per larva to cause more than 80.0 per cent larval mortality when the larvae of *G. mellonella* were exposed to nematodes for 8 hrs. The variation in the LD<sub>50</sub> value might be due to the variation in the period of exposure where in the present situation larvae of *G. mellonella* were exposed to juveniles of *S. carpocapsae* for 96 hrs.

#### **5.4.2 Pathogenicity of *S. carpocapsae* / *H. indica* on *Spodoptera litura***

Results pertaining to pathogenicity of nematodes on *S. litura* revealed that lethal infection was observed within 24 hrs at all dosages of both *S. carpocapsae* and *H. indica*. However, peak mortality of 73.20 per cent of *S. litura* by *S. carpocapsae* and 75.8 per cent by *H. indica* was recorded after 96 hrs when 80 IJS were inoculated. To get 50 per cent mortality of *S. litura*, 48 hrs of exposure to EPNs was required with an inoculum level of 80 IJS *S. carpocapsae* and 60 IJS in *H. indica*. Further, it was clear that LD<sub>50</sub> and LT<sub>50</sub> value for both nematodes were decreased with

increase in time and dosage respectively. Similar results were made by Arunaparihar *et al.* (2003) where they observed 50 per cent mortality within 72 hrs of exposure at an inoculum level of 100 IJS *Heterorhabditis*. The larval mortality was increased with increase in inoculum level of entomopathogenic nematodes and period of exposure. Present result also confirm with those of Hussaini *et al.* (2003) who observed highest larval mortality of *S. litura* at an inoculum level of 100 IJS of *Heterorhabditis* and 125 IJS of *Steinernema species*. In the present studies also high larval mortality of *S. litura* was observed at high inoculum level of 80 IJS per larva. However, these results are contrary to the observations of Prabhuraj *et al.* (2002) who concluded that  $LC_{50}$  for *S. glaseri* was 1165.2 IJS and for *H. minutus*, it was 4241.1 IJS per larva. In the present studies 50 per cent mortality of *S. litura* larvae was recorded within 48 hrs at both 60 and 80 IJS inoculum. Results obtained in the present studies are contrary to the report of Kang Young Lin *et al.* (2004), who observed corrected mortality of 53.3 – 66.7 per cent of larvae of *Spodoptera depravata* only after 5 days (120 hrs) of inoculation by *Heterorhabditis* and *S. carpocapsae*.

The early death of *Spodoptera litura* larva brought about by both *S. carpocapsae* and *H. indica* in the present studies might be due to variation in species of *Spodoptera* indicating the difference in pathogenicity of EPNs that resulted in different rate of mortality of the test insect.

#### **5.4.3 Pathogenicity of *S. carpocapsae* / *H. indica* on *Papilio demoleus***

Studies pertaining to effect of EPNs on *P. demoleus* showed that highest larval mortality of 96.0 per cent was observed after 96 hrs at a highest inoculum of 80 IJS per larva. Lowest mortality of 41.20 per cent was recorded at an inoculum level of 40 infective juveniles by both *S. carpocapsae* and *H. indica*. However, 50.0 per cent mortality of the larvae was obtained within 24 hrs. Present results are contrary to those of Singh (1993), who has recorded 45 per cent mortality of *P. demoleus* after 48 hrs and a cumulative mortality of 90 - 100 per cent after 10 days of inoculation of *S. carpocapsae* variation in the results might be due to variation in the stage of larval instar and as well as difference in exposure period to the juveniles of entomopathogenic nematodes.

## **CHAPTER – VI**

### **SUMMARY**

Studies related to survey for isolation of entomopathogenic nematodes in and around Tirupati region, their identification and mass multiplication, effect of environmental factors on nematode survival and pathogenicity and evaluation of entomopathogenic nematodes against important lepidopterous pests were carried out in insectary, S.V. Agricultural College, Tirupati during November to June, 2005.

For isolating entomopathogenic nematodes in and around Tirupati, soil samples were collected from January to June, 2005. Results revealed that no entomopathogenic nematodes could be recorded from any of the total 135 soil samples. Due to these reasons the parent cultures of EPNs of *S. carpocapsae* and *H. indica* were brought from P.D.B.C., Bangalore and carried out the research work.

Mass multiplication of the two entomopathogenic nematodes i.e. *S. carpocapsae* and *H. indica* was done using *G. mellonella*

larvae that yielded highest progeny as 2.30 lakhs per larva when 60 infective juveniles of *S. carpocapsae* were inoculated and lowest 1.49 lakh juveniles per larva recorded at an inoculum levels of 40 juveniles. In the case of *H. indica*, there was more high progeny production even with high inoculum level of 80 IJS per larva i.e. 4.34 lakh juveniles per larva. However, the same inoculum of *S. carpocapsae* could not yield high progeny under same conditions. It has recorded a lowest progeny of 3.52 lakh juveniles per larva at lowest inoculum level of 40 juveniles. It was clear from the present studies that a single *G. mellonella* larva can accommodate only 60 infective juveniles of *S. carpocapsae* and 80 juveniles of *H. indica* for mass multiplication. More than 60 infective juveniles of *S. carpocapsae* and 80 infective juveniles of *H. indica* could yield in low progeny.

With regard to the yield of entomopathogenic nematodes from different sized larvae of greater wax moth, *G. mellonella* results revealed that as the size of the larva increased it resulted in higher progeny production of both *S. carpocapsae* and *H. indica*. *S. carpocapsae* yielded high progeny of 1,02,400 juveniles per larva from large sized larva (body size of 20 – 22 mm and weight of

350 mg), whereas small sized larva (body size of 10-12 mm and weight of 150 mg), yielded lowest progeny of 81,340 juveniles per larva. *H. indica* also yielded high progeny of 2,01,560 juveniles from large sized larva (size of 20-22 mm and weight of 340 mg), whereas the small sized larva (body size of 12-14 mm and weight of 140 mg) yielded only 1,06,000 juveniles per larva.

Estimation of progeny production of the entomopathogenic nematodes tested on different insect hosts i.e. *G. mellonella*, *C. cephalonica* and *B. mori* revealed that *G. mellonella* yielded highest progeny of *S. carpocapsae* 2.3 lakh infective juveniles per larva followed by *C. cephalonica* with 1.83 lakh IJS per larva and lowest of 1.13 lakh IJS in *B. mori*. Similar pattern of progeny yield was recorded with respect to *H. indica* i.e. *G. mellonella* yielded high population of 4.1 lakhs juveniles per larva followed by 2.52 lakh juveniles from *C. cephalonica*, where as lowest of 2.13 lakhs population by *B. mori*. It was clear from the results that *G. mellonella* is the best host that could be used for mass production of *S. carpocapsae* and *H. indica* and *C. cephalonica* form the alternate host in the absence of *G. mellonella*.

Studies on the effect of temperature on survival of the entomopathogenic nematodes made under laboratory conditions revealed that 25°C temperature was the optimum temperature, showed a maximum of 95.82 per cent survival of the infective juveniles of the *S. carpocapsae*. Temperature of 35°C was found to be unfavourable resulted the lowest 37.14 per cent survival of the infective juveniles. When temperature was decreased to 20°C *S. carpocapsae* recorded 84.57 per cent survival and at 25°C *H. indicia* recorded only 88.89 per cent survival of infective juveniles. A temperature of 20°C was recorded as optimum showing maximum of 91.66 per cent survival of infective juveniles. 35°C was most unfavourable which resulted only 40.30 per cent survival of the infective juveniles. However, for both the nematodes, 35°C temperature was found to affect survival showing only 37.14 per cent in *S. carpocapsae* and 40.30 per cent in the case of *H. indica*.

Studies pertaining to know the effect of temperature on nematode pathogenicity against *G. mellonella*, revealed that among four levels of temperature (i.e. 20, 25, 30 and 35°C), 25°C was recorded as optimum temperature which resulted in 100 per cent

mortality of the larva by both the nematode species i.e. *S. carpocapsae* and *H. indica*. Temperature of 35°C was proved to be the most unfavourable temperature for causing infection of *G. mellonella* larva by *S. carpocapsae* and *H. indica* which has resulted the lowest mortality of the larvae i.e. 31.05 and 30.03 per cent respectively.

Influence of relative humidity on the survival of the infective juveniles revealed that increased relative humidity improved the activity of the juveniles of both *S. carpocapsae* and *H. indica*. About 80 per cent relative humidity resulted maximum survival of 79.72 per cent in *S. carpocapsae* and 79.50 per cent in *H. indica*. Whereas 50 per cent relative humidity resulted in lowest survival of 4.17 per cent of the infective juveniles of *S. carpocapsae* and 7.0 per cent of *H. indica*.

Studies on effect of relative humidity on nematode pathogenicity revealed that there was a linear relationship between relative humidity and larval mortality, where a significant increase in mortality of *G. mellonella* larva was observed at highest relative humidity of 80 per cent showing 78.10 per cent by *S. carpocapsae*

and 81.58 per cent by *H.indica* . At lowest relative humidity of 50 per cent was recorded only 41.69 per cent larvae mortality by *S. carpocapsae* and 50.00 per cent by *H. indica*.

Experiment on effect of U.V. radiation on survival of the entomopathogenic nematodes revealed that if the juveniles were exposed to U.V. radiation (254 nm) for a longer period of 120 minutes, it could result in complete mortality of juveniles of both *S. carpocapsae* and *H. indica*. It could be concluded from the present studies that among the two nematodes this could result in 100.00 per cent mortality of *H. indica* with in 90 minutes of U.V. light exposure.

Results pertaining to effect of U.V radiation on nematode pathogenicity revealed that *S. carpocapsae* was more virulent in causing mortality of the test insect when infected with juveniles that were exposed to U.V. light for 75 minutes. In the case of *H. indica*, the juveniles when exposed to U.V. radiation for more than 60 minutes, they could not infect the *G. mellonella* larvae. A maximum of 15 minutes exposure of IJS of *S. carpocapsae* and

*H. indica* could cause 100 per cent mortality of the test insect, after which it could affect the pathogenicity.

Experiment on influence of pH on nematode survival revealed that a gradual increase in percentage survival of infective juveniles of *S. carpocapsae* and *H. indica* with increasing pH from 5 to 7 was observed and with increasing pH from 7 to 9 decreasing survival was observed. pH of 7, maximum survival of 48.55 per cent *S. carpocapsae* and 55.12 per cent of *H. indica* was recorded. Very low survival of 16.8 per cent *S. carpocapsae* and 21.03 per cent of *H. indica* was recorded at a pH level of 9.

In addition to survival, effect of pH on nematode pathogenicity revealed that nematode activity and its ability in causing the mortality of the test insect remained unaltered at neutral pH of 7 recording high larval mortality of 78.6 per cent by *S. carpocapsae* and 82.06 per cent by *H. indica*. A slight deviation of pH from 7 to 5 or from 7 to 9 could result in less than 50.0 per cent mortality of *G. mellonella* by both *S. carpocapsae* and *H. indica*.

Studies on the effect of soil moisture on survival of the nematodes revealed that there was a gradual increase in percentage survival of the juveniles with increasing soil moisture from 5 to 15 per cent (field capacity level) and later there was a decrease in survival of IJS when soil moisture was further increased to 25 per cent. Hence, it was concluded that 15 per cent moisture level was suitable level for both *S. carpocapsae* and *H. indica* that could show maximum survival of 78.62 and 80.41 per cent respectively. Twenty per cent moisture level was unfavourable for survival of the two nematode species showing lowest survival of 39.58 per cent of *S. carpocapsae* and 43.72 per cent of *H. indica* was recorded.

With respect to effect of soil moisture on pathogenicity of two entomopathogenic nematode species i.e. *S. carpocapsae* and *H. indica* revealed that activity and pathogenicity by both nematode species was found high at 15 per cent moisture level showing larval mortality of 75.97 per cent by *S. carpocapsae* and 79.99 per cent by *H. indica*. At 25 per cent moisture level, there was low larval mortality of 35.88 and 43.78 per cent by *S. carpocapsae* and *H. indica* respectively was observed.

Impact of soil type on nematode pathogenicity revealed that among three types of soils black soil was more suitable for nematode activity as well as for causing mortality of the larvae of *G. mellonella*, which was recorded as 72.60 per cent by *S. carpocapsae* and 77.28 per cent by *H. indica*, followed by sandy loam, showing 67.33 per cent by *S. carpocapsae* and 67.86 per cent by *H. indica*. Lowest of 58.20 per cent mortality by *S. carpocapsae* and 64.06 per cent by *H. indica* was recorded in sandy soil. Results pertaining to the depth of soil revealed among three depths of soil a depth of 5 cm was optimum that could result in high larval mortality ranging from 77.33 to 90.70 per cent in all types of soils by both *S. carpocapsae* and *H. indica*.

Studies made on efficacy of the two entomopathogenic nematodes i.e. *S. carpocapsae* and *H. indica* against the control of three lepidopteran pests i.e. *H. armigera*, *S. litura* and *P. demoleu* revealed that among the three pests *P. demoleus* was more susceptible recording a maximum mortality of 75.73 per cent by *S. carpocapsae* and 70.45 per cent by *H. indica* followed by *H. armigera* showing 66.65 per cent by *S. carpocapsae* and 67.95 per cent *H. indica*. *S. litura* recorded a maximum mortality of

54.93 per cent by *S. carpocapsae* and 56.95 per cent *H. indica*. It was also clear that with increasing level of inoculum, there was a gradual increase in larval mortality. With regard to the median lethal dose for the LD<sub>50</sub> for *P. demoleus* was low compared to *H. armigera* and *S. litura*. It was recorded as LD<sub>50</sub> of 26.55 IJS of *S. carpocapsae* and 26.22 IJS of *H. indica* for *P. demoleus* followed by 28.67 IJS of *S. carpocapsae* and 28.89 IJS of *H. indica* for *S. litura* and 35.24 IJS of *S. carpocapsae* and 37.30 IJS of *H. indica* for *H. armigera* for getting 50 per cent mortality with in 96 hrs. The lethal time recorded was 21.63 hrs of *S. carpocapsae* and 11.43 hrs for *H. indica* for *P. demoleus* followed by 23.55 hrs of *S. carpocapsae* and 21.58 hrs of *H. indicia* for *H. armigera* and 42.95 hrs of *S. carpocapsae* and 31.05 hrs of *H. indica* for *S. litura* were required for getting 50 per cent mortality at 80 IJS level.

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