# “ISOLATION, IDENTIFICATION AND MASS MULTIPLICATION OF ENTOMOPATHOGENIC NEMATODES (EPNs) AT DIFFERENT DISTRICTS OF CHHATTISGARH”

**M.Sc. (Ag.) THESIS**

**By**

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**DEPARTMENT OF ENTOMOLOGY**
**COLLEGE OF AGRICULTURE FACULTY OF AGRICULTURE**
**INDIRA GANDHI KRISHI VISHWAVIDYALAYA**
**RAIPUR (CHHATTISGARH)**
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# “ISOLATION, IDENTIFICATION AND MASS MULTIPLICATION OF ENTOMOPATHOGENIC NEMATODES (EPNs) AT DIFFERENT DISTRICTS OF CHHATTISGARH”

**Thesis**

**Submitted to the**

**Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)**

**By**

**Nistha Tiwari**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF**

**Master of Science**
**in**
**Agriculture**

**(ENTOMOLOGY)**

**ID No. 20161724870** **Roll No. 120116114**

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## CERTIFICATE – I

This is to certify that the thesis entitled "**Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh**" submitted in partial fulfilment of the requirements for the degree of "**Master of Science in Agriculture**" of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Nistha Tiwari** under my/our guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

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

**Date:** **Chairman**

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### THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Chairman: Dr. Jaya Laxmi Ganguli ____________

Member: Dr. V.K. Dubey ____________

Member: Dr. Shubha Banerjee ____________

Member: Dr. Ravi R. Saxena ____________

## CERTIFICATE – II

This is to certify that the thesis entitled **"Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh"** submitted by **Nistha Tiwari** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture** in the **Department of Entomology** has been approved by the external examiner and Student's Advisory Committee after oral examination.

**Date:**

**Signature External Examiner**

**(Name** Dr. Shashidhar Viraktamath**)**

**Major Advisor** ____________________

**Head of the Department** ____________________

**Dean/ Dean Faculty** ____________________

**Director of Instructions** ____________________

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*Department of Entomology* **_Nistha Tiwari_**

*College of Agriculture, IGKV, Raipur*

*Date:*

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# LIST OF NOTATIONS / SYMBOLS

| SYMBOLS | | DESCRIPTION |
|---|---|---|
| **%** | : | Percent |
| **@** | : | At the rate of |
| **$^{o}$C** | : | Degree Celsius |

# LIST OF ABBREVIATIONS

| ABBREVIATIONS | | DESCRIPTION |
|---|---|---|
| EPNs | : | Entomopathogenic nematodes |
| IJs | : | Infective juveniles |
| *S. carpocapsae* | : | *Steinernema carpocapsae* |
| *H. indica* | : | *Heterorhabditis indica* |
| *H. bacteriophora* | : | *Heterorhabditis bacteriophora* |
| *G. mellonella* | : | *Galleria mellonella* |
| *C. cephalonica* | : | *Corcyra cephalonica* |
| *H. armigera* | : | *Helicoverpa armigera* |
| *S. litura* | : | *Spodoptera litura* |
| Species | : | Species |
| U.V radiation | : | Ultraviolet radiation |
| pH | : | Potential of Hydrogen |
| Min. | : | Minimum |
| Max. | : | Maximum |
| Sr. | : | Serial |
| No. | : | Numbers |
| Hrs. | : | Hours |
| Fig. | : | Figure |
| Avg. | : | Average |
| *i.e.* | : | That is |
| *et.al.* | : | and others |
| mg | : | Milligram |
| min. | : | Minutes |
| ml | : | Millilitre |
| mm | : | Millimeter |

| | | |
|---|---|---|
| nm | : | Nanometer |
| cm | : | Centimeter |
| *vi*z. | : | Namely |
| NS | : | Non-Significant |
| CD | : | Critical difference |

## THESIS ABSTRACT

| | | |
|---|---|---|
| a) Title of the Thesis | : | Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh |
| b) Full Name of the Student | : | Nistha Tiwari |
| c) Major Subject | : | Entomology |
| d) Name and Address of the Major Advisor | : | Dr. (Smt.) Jaya Laxmi Ganguli<br>Professor, (Entomology),<br>College ofAgriculture, Indira Gandhi KrishiVishwavidyalaya, Raipur, (C.G.) |
| e) Degree to be awarded | : | Master of Science in Agriculture (Entomology) |

Signature of Student

**Signature of Major Advisor**

**Date: _____________**

Signature of Head of the Department

## ABSTRACT

In the present studies, two species of EPNs *viz. Steinernema carpocapsae* (Weiser) and *Heterorhabditis indica* Poinar, Karunakar and David were isolated and identified from 82 soil samples collected during 2017-18 from various agro-ecosystems belonging to different districts at three agro-climatic zones of Chhattisgarh. Both the isolates were isolated from the loamy soil. *S. carpocapsae* isolates were identified from yam ecosystem at Raipur district and *H. indica* isolates from the rose ecosystem of Durg district.

Studies conducted on the effect of temperature on survival of the EPNs under laboratory conditions revealed that the temperature of 25°C was found to be the optimum temperature, with maximum 99.60 per cent survival of IJs of *S. carpocapsae*. Higher temperature of 35°C was found to be unfavourable as it resulted in lowest 44.93 per cent survival of the IJs. For *H. indica* also temperature of 25°C was recorded as optimum, showing maximum of 99.95 per cent survival of IJs. Higher temperature of 35°C was most unfavourable which resulted in only 46.40 per cent survival of the IJs.

Studies on the effect of U.V. radiation on survival of *S. carpocapsae* and *H. indica* recorded 86.12 and 82.37 per cent survival of IJs respectively up to 15.00 minutes of U.V radiation exposure (254 nm). Long periods of exposure *i.e.* 105.00 and 120.00 minutes resulted in complete mortality of IJs of both the species. It was found that *S. carpocapsae* could comparatively resist U.V radiation for longer period than *H. indica*.

Result on the effect of pH on survival of the IJs of *S. carpocapsae* indicated a gradual increase in percentage survival of IJs with increasing pH from 4.00 to 7.00. Highest survival was found at neutral of pH 7.00 *i.e.* 99.31 per cent of the IJs of *S. carpocapsae* followed by 98.93 and 98.75 at alkaline pH 9.00 and 8.00 respectively, and lowest survival was found at acidic pH 4.00 *i.e.* 96.43 per cent. But in case of IJs of *H. indica* highest survival was found at pH 5.00 *i.e.* 88.81 per cent followed by 85.50 per cent at pH 6.00 which reduced to 81.93 per cent at pH 7.00 and lowest survival was observed at pH 4.00 *i.e.* 62.12 per cent. Thus, pH 7.00 was found to be the optimum level of pH for maximum survival of the *S. carpocapsae* and could go well with increase in pH als0 while pH 5.00 was most suitable for *H. indica.*

In the experiment conducted on progeny production, *G. mellonella* harvested 94,311.66 and 1,68,234.83 IJs / larva of *S. carpocapsae* and *H. indica* respectively. In case of *C. cephalonica* progeny production of *S. carpocapsae* and *H. indica* 84,484.83 IJs and 1,09,094.83 IJs/larva respectively. The length and body weight of larvae were found to be directly proportional with the progeny production of IJs as the length and body weight of *G. mellonella* and *C. cephalonica* increased progeny production of both the IJs of *S. carpocapsae* and *H. indica* also increased.

Mass multiplication of the two EPNs *i.e. S. carpocapsae* and *H. indica* was done using *G. mellonella* larvae and yielded highest progeny of 2,42,119.50 IJs per larva at the dose of 60 IJs of *S. carpocapsae* and lowest progeny of 1,20,957.00 IJs per larva was recorded at 20 IJs inoculum. In the case of *H. indica* highest progeny harvested was of 4,82,084.50 and lowest progeny of 3,24,269.50 IJs per larva with an inoculums doses of 80 and 20 IJs respectively.

Studies on the mass multiplication of the two species of EPNs *i.e. S. carpocapsae* and *H. indica* using *C. cephalonica* larvae that yielded highest progeny of 1,65,388.80 IJs per larva at 60 IJs inoculum and lowest 88,389.50 IJs per larva recorded at 20 IJs inoculum of *S. carpocapsae* . In the case of *H. indica*, highest progeny harvested 3,86,737.00 and lowest of 2,02,735.50 was harvested at 80 and 20 IJs of inoculums respectively.

Estimation of progeny production of the two species of EPNs tested on four different lepidopteran larvae as hosts *i.e. G. mellonella, C. cephalonica*, *H. armigera* and *S. litura* revealed that *G. mellonella* yielded highest progeny of *S. carpocapsae* 1,64,098.80 IJs/ larva followed by *C. cephalonica* with 99,126.75 IJs per larva and lowest of 88,609.75 and 77,849.25 IJs/ larva in *H. armigera* and *S. litura* respectively. Similar pattern of progeny yield was recorded in *H. indica* also, where *G. mellonella* yielded highest population of 3,42,264.00 IJs/ larva followed by 1,22,181.30 IJs/ larva from *C. cephalonica*, where as lowest of 1,02,874.80 and 89,174.75 IJs/ larva were obtained in *H. armigera* and *S. litura* respectively. Thus, it is was clear from the results that *G. mellonella* proved to be the best host that could be used for mass production of *S. carpocapsae* and *H. indica* and *C. cephalonica* could be the alternate host in absence of *G. mellonella.*

## भाोध सारांश

| | | |
|---|---|---|
| अ) शोध का शीर्षक | : | “छत्तीसगढ़ के विभिन्न जिलों से कीट रोगकारी सूत्रकृमि का पृथककरण, अभिनिर्धारण एवं संवर्धन ” |
| ब) छात्रा का पूरा नाम | : | निष्ठा तिवारी |
| स) मुख्य विषय | : | कीट विज्ञान |
| द) मुख्य सलाहकार का नाम और पता | : | डॉ. (श्रीमती) जयालक्ष्मी गांगुली, मुख्य सलाहकार, प्राध्यापक (कीट विज्ञान), कृषि महाविद्यालय, इंदिरा गांधी कृषि वि”वविद्यालय, रायपुर, (छ.ग.) |
| इ) उपाधि का नाम | : | एम.एस.सी. (कृषि) |

छात्रा का हस्ताक्षर

मुख्य सलाहकार के हस्ताक्षर
दिनांक..............................

विभागाध्यक्ष के हस्ताक्षर

## भाोध सारांश


वर्तमान अध्ययन में  कीट रोगकारी सूत्रकृमि की दो प्रजातियाँ क्रम”ाः *स्टीनरनेमा कार्पोकैप्सी* और *हेटेरोर्हब्डिटीस इंडिका* को छत्तीसगढ के तीनो कृषि जलवायु क्षेत्रों के विभिन्न जिलों से संबंधित कृषि–फसल पारिस्थितिकी तंत्र से 2017–18 के दौरान संग्रहण किए गए 82 नमूनों में से पृथक एवं अभिनिर्धारण (पहचान) किया गया। दोनों प्रजातियाँ दोमट मिट्‌टी से पृथक किए, जिसमें *एस. कार्पोकैप्सी* रायपुर के जिमीकन्द कृषि–फसल पारिस्थितिकी तंत्र एवं *एच. इंडिका* गुलाब फसल पारिस्थितिकी तंत्र दुर्ग से पृथक किया गया।

बायोकंट्रोल प्रयोग”ााला में तापमान के प्रभाव का अध्ययन, कीट रोगकारी सूत्रकृमि के जीविका दर पर किया गया, जिससे ज्ञात हुआ कि $25^{0}$ से. का तापमान

इष्टतम तापमान था, जिसमें *एस. कार्पोकैप्सी* की अधिकतम 99.60 प्रति"ात जीविका दर थी एवं 35$^{o}$ से. पर प्रतिकुल प्रभाव पायी गई जो कि 44.93 प्रति"ात दर्ज की गई। इसी प्रकार *एच. इंडिका* के लिए भी 25$^{o}$ से. का तापमान उत्तम पायी गई जिसमें अधिकतम 99.95 प्रति"ात जीविका दर एवं 35$^{o}$ स. पर प्रतिकुल प्रभाव, जिसके परिणामस्वरूप केवल 46.40 प्रति"ात जीविका दर रही।

पराबैंगनी विकिरण के प्रभाव का अध्ययन *एस. कार्पोकैप्सी* एवं *एच. इंडिका* के जीविका दर पर किया, जिसमें 15:00 मिनट तक पराबैंगनी विकिरण के प्रभाव में अधिकतम 86.12 एवं 82.37 प्रति"ात जीविका दर एवं लंबी अवधि तक पराबैंगनी के संपर्क में प्रतिकुल प्रभाव देखा गया, जिसके फलस्वरूप 120:00 एवं 105:00 मिनटों में दोनों सूत्रकृमियों की पूर्णतः मृत्यु हो गई। इससे यह स्पष्ट होता है कि *एस. कार्पोकैप्सी, एच. इंडिका* की तुलना में अधिक समय तक पराबैंगनी विकिरण का प्रतिरोधक है।

पी.एच. के प्रभाव का अध्ययन *एस. कार्पोकैप्सी* के जीविका दर पर किया गया, जिसके परिणामस्वरूप पी.एच. मान 4.00 से 7.00 तक बढ़ने पर सूत्रकृमि जीविका दर में क्रम"ाः वृद्धि हुई एवं पी.एच. 7.00 पर अधिकतम 99.31 प्रति"ात जीविका दर पायी गई तथा 98.93 और 98.75 प्रति"ात क्रम"ाः क्षारीय पी.एच. 9.00 और 8.00 पर पाया गया। न्यूनतम जीविका दर पी.एच. 4.00 पर 96.43 प्रति"ात दर्ज किया गया। इस प्रकार एच. इंडिका में सबसे अधिक जीविका दर पी.एच. 5.00 पर 88.81 प्रति"ात देखा गया जिसके बाद पी.एच. 6.00 पर 85.50 प्रति"ात एवं पी.एच. 7.00 घटकर 81.93 प्रति"ात और न्यूनतम जीविका दर पी.एच. 4.00 पर 62.12 प्रति"ात दर्ज किया गया। *एस. कार्पोकैप्सी* की अधिकतम जीविका दर पी.एच. 7.00 पर एवं पी.एच. 5.00 पर *एच. इंडिका* के जीविका दर के लिए अनुकुल पाया गया।

संवर्धन के प्रयागे हेतु मोम शलभ, *गैलेरिया मेलोनेला* का उपयोग किया, जिसमें *एस. कार्पोकैप्सी* एवं *एच. इंडिका* की संतति 94,311.66 और 1,68,234.83 प्रति इल्ली क्रम"ाः प्राप्त किया गया। इसी प्रकार चावल के शलभ, *कोरसाइरा सिफालोनिका* का भी

प्रयोग संतति उत्पत्ति 84,484.83 एवं 1,09,094.86 प्रति इल्ली क्रम"ाः प्राप्त हुआ। इल्ली की शरीर की लम्बाई एवं वजन के अनुसार संवर्धन में सीधी वृद्धि पाई गई।

रोगकारी सूत्रकृमि *एस. कार्पोकैप्सी* एवं *एच. इंडिका* की संतति उत्पत्ति हेतु *गैलेरिया मेलोनेला* का प्रयोग किया गया, जिसमें *एस. कार्पोकैप्सी* की अधिकतम 60.00 एवं न्यूनतम 20.00 मात्रा डालने पर संतति उत्पत्ति क्रम"ाः 2,42,119.50 एवं 1,20,957.00 प्राप्त हुआ। इसी प्रकार *एच. इंडिका* की अधिकतम 80.00 एवं न्यूनतम 20.00 मात्रा डालने पर संतति उत्पत्ति क्रम"ाः 4,82,083.50 एवं 3,24,269.50 प्रति इल्ली प्राप्त किया गया।

रोगकारी सूत्रकृमि *एस. कार्पोकैप्सी* और *एच. इंडिका* की संतति उत्पत्ति हेतु *कोरसाइरा सिफालोनिका* का भी प्रयोग किया गया, जिसमें *एस. कार्पोकैप्सी* की अधिकतम 60.00 एवं न्यूनतम 20.00 मात्रा डालने पर संतति उत्पत्ति क्रम"ाः 1,65,388. 80 एवं 88,389.50 प्राप्त हुई। इसी प्रकार *एच. इंडिका* की अधिकतम 60.00 एवं न्यूनतम 20.00 मात्रा डालने पर संतति उत्पत्ति क्रम"ाः 3,86,737.00 एवं 2,02,735.50 प्रति इल्ली प्राप्त की गई।

रोगकारी सूत्रकृमि दोनों प्रजातियों की संतति उत्पत्ति हेतु लेपिडोपटरा परिवार के चार अलग–अलग प्रजातियों की इल्लियों पर परीक्षण किया गया जैसे कि *गैलेरिया मेलोनेला, कोरसाइरा सिफालोनिका, हेलिकोवेरपा आर्मिजेरा, स्पोडोप्टेरा लिटुरा,* जिससे यह ज्ञात हुआ कि *एस. कार्पोकैप्सी* की उच्चतम संतति *जी. मेलोनेला* से 1,64,098.80 प्रति इल्ली से प्राप्त हुई। उसके प"चात् 99,126.75 संतति *सी. सिफालोनिका* से एवं 88,609.75 और 77,849.25 संतति प्रति इल्ली *एच. आर्मिजेरा* और *एस. लिटुरा* से क्रम"ाः प्राप्त किया गया। इसी प्रकार *एच. इंडिका* की भी सर्वाधिक संतति उत्पत्ति *जी. मेलोनेला* से 3,42,264.00 प्रति इल्ली प्राप्त हुआ। ततप"चात् 1,22,181.30, 1,02,874.80 और 89,174.75 संतति क्रम"ाः *सी. सिफालोनिका, एच. आर्मिजेरा* और *एस. लिटुरा* से प्राप्त की गई।

# CHAPTER - I
# INTRODUCTION

Nematodes are microscopic, multicellular, non-segmented, elongated body and colorless roundworms with no appendages. They are ubiquitous in nature and diverse in habitat. More than 28,000 nematodes species have been described and global richness of species that has been estimated between five lakhs to one million (Hugot *et al.* 2001). Nematodes are present in terrestrial regions, fresh water conditions and also marine environments. Lifestyles of some nematodes are free-living and many are parasites of plants, animals including humans. They are emerging as powerful model for studying the different disciplines such as physiology, ecology, biology and evolution. Nematodes that have ability to cause infection and kill insects within 24 to 48 hours are known as "Entomopathogenic nematodes" (EPNs)

EPNs are roundworms with non-segmented and soft body that are facultative as well as obligate parasite of insects, naturally occuring in soil conditions and locate the host through response of chemicals, vibrations and $CO_2$ (Kaya and Gaugler 1993). Both the EPNs, that belong to families Steinernematidae and Heterorhabditidae have been successfully used as biological agents in effective pest management program (Grewal *et al.* 2005). These two families of EPNs have many similar character such as their symbiotic association with enterobacteria (Enterobacteriaceae family and genera *Xenorhabdus* and *Photorhabdus)* present in their intestine and they also mutually share their need to infect the host.

EPNs and their associated bacteria cause disease to a wide range of hosts and have been effectively used as biological control agents in integrated pest management programs worldwide (Duncan and McCoy, 1996; Gaugler *et al.* 1997; Lacey and Unruh, 1998; Shapiro and McCoy, 2000). Agriculturally important insect pests, *i.e. Amyelois transitella* in almond (Rice *et al.* 1978), crickets (Parkman *et al.* 1996), scarab beetles (Koppenhofer and Kaya, 1997) flower thrips (Ebbsa *et al.* 2001) and fungus gnats (Jagdale *et al.* 2004) have been successfully managed by EPNs. *Steinernema carpocapsae* used against *Helicoverpa armigera and Spodoptera litura,* (Gupta *et al.* 1987) *S. feltia* and *S. carpocapsae* against *H. armigera* (Ghode *et al.* 1988) *H. bacteriophora* against *Coryra cephalonica* (Sivakumar *et al.* 1989) *S.*

*carpocapsae* DD136 *against Holotrichia consanguinea* (Shanthi and Sivakumar, 1991) have been successfully deployed.

As per Gaugler and Kaya (1990) the infective juveniles stage (IJs) are the only free living stage which vectors the symbiotic bacteria, carrying it from one host insect to other. After searching a suitable host in soil, the IJs enter inside the body of host insects through the mouth, respiratory exterior openings *i.e.* spiracles or through anus and finally finds its way into the haemocoel (Kaya, 1993). EPNs *viz., Heterorhabditis* spp. have buccal cuticular tooth through which they penetrate the insect cuticle (Bedding and Molyneux, 1982). When the IJs reach in the host bodies, then the bacteria are released into the haemocoel, and after 24 to 48 hours kills the host due to excessive septicemia. Thus, one to three generations of EPNs may develop inside the host by consuming symbiotic bacteria and degraded tissues and the new generations form colonies inside the carcass. After the food gets exhausted, the newly formed IJs leave the old cadaver in search of new hosts (Poinar, 1990).

Studies under laboratory conditions, showing broad ranges of host for EPNs because in this condition, host contact is assured and their surroundings are also ideal and no such "ecological barriers" for causing disease (Kaya and Gaugler 1993, Gaugler, 1997). Thus, the wide host ranges predicted initially by assay outcomes have not always been translated into pesticidal goals.

EPNs of the genera *Steinernema* and *Heterorhabditis* easily mass multiplication on the fifth instar of greater wax moth larvae *i.e. Galleria mellonella* (L.) and rice moth *i.e Corcyra cephalonica* (S.). IJs are sensitive to extreme of physical environmental conditions *i.e.* temperature, pH, UV radiation, relative humidity and moisture level they are inactivated in such conditions and prevent them for this extremes. The various species and isolates showed differences in behavior, infectivity, reproduction, host ranges and environmental tolerances.

This biodiversity of EPNs has been initiated interest in characterization of the genetic variations as new species and strains may have such different ecological or biological character that would be more beneficial than those recently used as biological control agents against important agricultural insects (Oestergaard *et al.* 2006, Shapiro-Ilan *et al.* 2006, Vinciguerra and Clausi, 2006 and El-Borai *et al.* 2007). All these aspects, led to realization of the practical use of EPNs and far broader scientific view on the spurred development and for focussing on molecular characterization of EPNs. Searching of new EPN species worldwide, has resulted in an exponential growth and all over more than thousands of new isolates have been discovered.

According to Campbell and Gaugler (1997) the foraging habits of EPNs vary between species, depending on the depth of soil, distribution and host preferences. EPNs search for their hosts by two ways, either as ambusher or as cruiser. Many *Steinernema* spp. shows ambusher nature and have ablity to form loops which propels their body with stored energy and other *Heterorhabditis* spp. adopt cruiser habits. Instead of searching for potential hosts they roam through the soil. For example, ambushers infect more insects on the surface like *S. carpocapsae*, while cruisers infect insects deep in the soil like *H. bacteriophora* (Campbell and Gaugler 1993).

The soil texture affects the ability of EPNs to infect the host and also affect their horizontal and vertical dispersal. The pore space of soil is determined by the arrangement of soil particles and concentration of oxygen level. EPNs showed better per cent survival in sandy loam soil than in clay soil. The poor survival per cent in clay soil is due to lower concentration of oxygen present in the smaller sizes of pore space of soil. Oxygen becomes a limiting factor in soils with high organic content and water saturated condition.

Chhattisgarh state is bestowed with a rich flora and fauna under three agro-climatic zones, namely Northern hills, Chhattisgarh plains and Bastar plateau. All these zones differ considerably in their climatic conditions, soil types such as Bhata (Entisol), Matasi (Inceptisol), Dorsa (Alfisol), Kanhar (Vertisol) and least presence of Molisol and various cultivation practices, leading to diversified crop agro-ecosystems. So far, practically no work has been done on EPNs, their isolation, identification, biological variation and mass multiplication.

Looking to the above facts, the present research work was formulated entitled **"Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh"** which was carried out in the Biological control laboratory, Department of Entomology, College of Agriculture, IGKV, Raipur under the following objectives:-

**Objectives:-**

1. To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.
2. To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh.
3. To study the mass multiplication of EPNs at laboratory condition on different hosts.

# CHAPTER - II

# REVIEW OF LITERATURE

In this chapter, review of literature pertaining to **"Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh"** was carried out by several researchers in Chhattisgarh, India and abroad that has been mentioned. The literature on different aspects is briefly reviewed under the following objectives:

1. To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.
2. To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh.
3. To study the mass multiplication of EPNs at laboratory condition on different hosts.

## 2.1 To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.

Kung *et al.* (1990a) reported that out of 84 selected sites, collected the 280 soil samples from natural vegetation and agriculture land were positive for EPNs 32 and 26 samples *i.e.* 11.40 and 31.00 per cent respectively, in Guinean zone of Southern Benin. Two species were isolated *i.e*. *Heterorhabditis indica* and *H. sonorensis.*

Gaugler *et al.* (1992) surveyed 304 sites in New Jersey for collection of soil samples in geographically and ecologically diverse area. Whereas, 66 soil samples *i.e*. 21.71 per cent were reported positive for EPNs in which 24 isolates of *Steinernema* spp. and 42 isolates of *Heterorhabditis* spp. The most commanly isolated species was *H. bacteriophora* where found 38 isolates, followed by *S. glaseri*, *S. feltiae*, *S. carpocapsae*, *Heterorhabditis* spp. and *Steinernema* spp. were 14, 4, 4, 4 and 2 isolates respectively.

Liu and Berry (1995) collected 255 soil samples from 59 different sites which from ecologically and geographical diverse habitats in the state of Oregon (US). EPNs were isolated from thirty samples of fourteen different sites. *Heterorhabditis* spp. and *Steinernema* spp. isolated from twenty four and seven samples collected from ten and five different sites respectively.

Kumar and Sivakumar (1997) surveyed the Vilarancode kulkulm and Kanyakumari for isolation of EPNs and reported isolates of *Steinernema* spp. and *H. indica* from collected seventeen and one sample respectively, out of 163 samples. Availability of *Steinernema* spp. was more in hilly areas with heavy rainfall and also more found in loamy soils.

Stock *et al.* (1999) analyzed 270 soil samples collected from 30 different sites in 10 geographical regions of California, and examined the presence of EPNs. They were positively isolated the samples of 26.3 per cent. These isolates were identified as *S. feltiae, S. carpocapsae*, *S. kraussei*, *H. bacteriophora* and *H. marelatus*.

Kaushal *et al.* (2000) collected 207 soil samples from different habitats, *viz*., Uttar Pradesh, Himachal Pradesh, Gujarat, Assam and Delhi, whereas, only seventeen samples *i.e.* 8.21 per cent contained positively EPNs, from 10 isolates of *Steinernema* spp. and 7 isolates of *Heterorhabditis* spp. respectively.

Hussaini *et al.* (2001) surveyed the throughout state of Karnataka for collection of soil samples and isolation of EPNs. They were isolated some isolates such as *H. indica* and *S. carpocapsae*, when the atmospheric temperature was less than 25°C.

Rajkumar *et al.* (2001) examined 105 soil samples collected from Udaipur district of Rajasthan, out of 105 samples, five samples were found positive to EPNs *i.e. Steinernema* spp.

Ambika and Sivakumar (2002) surveyed for isolation of EPNs in the northern parts of Coimbatore, Nilgiris and Erode, in between May 1994 to February 1995. Overall, from twenty different sites collected 171 soil samples. Out of 171 samples, 28 samples showed positive to EPNs *viz, H. indica* and *Steinernema* spp. with frequency of occurrence as per cent of 16.37.

Parihar *et al.* (2002) isolated EPNs *viz., Steinernema* spp. and *Heterorhabditis* spp. from 13 different districts of Rajasthan. Total 477 collected soil samples, out of which *Steinernema* spp. was found only in three samples of Udaipur. Whereas, *Heterorhabditis* spp. was found in one and four samples from Jaipur and Udaipur districts, respectively.

Eswarmoorthy and Sankaranarayanan (2003) reported isolates of *Heterorhabditis indica* isolated from the infected cadaver of sugarcane top borer *Scirpophaga excerptalis* in the vicinity of Coimbatore.

Hazir *et al.* (2003) isolated and identified of EPNs from different sites of Turkey where positive sites for each isolates of 2.03 per cent. EPNs isolated from fifteen samples were *Steinernema* spp. and seven were *Heterorhabditis* spp. Based on morphological and molecular characterization, the commonly identified species of EPNs was *H. bacteriophora, S. affine, S. feltiae*, and *Steinernema* spp. The new species of *Steinernema* was found from grassland.

Prasad and Katti (2003) conducted random survey for collected soil samples from 20 localities of Hyderabad. EPNs were isolated through *Corcyra* baiting technique whereas, three samples larvae resulted postive symptoms of EPNs infection out of twenty samples larvae. The mortality of larvae recorded within 10 to 20 hours after baiting technique.

Shapiro-Ilan *et al.* (2003) collected soil samples from 105 sites of 21 orchards at Arkansas, Louisiana, Mississippi and Georgia in the South Eastern United States. EPNs were isolated with infested *C. caryae* and *Galleria mellonella* where six isolates were recorded.

Uribe-Lorio *et al.* (2005) surveyed of isolation of EPNs in the Southeast Caribbean (Gandoca-Manzanillo Natural Refuge) regions of Costa Rica and North Pacific (Guanacaste Conservation Area) were reported that EPNs isolated from five samples of 20.5 per cent, out of 41 samples in which three samples of 12.30 per cent and two samples of 8.2 per cent containing *Steinernema* and *Heterorhabditis* isolates respectively.

According to the Anonymous (2006) surveyed for isolation of EPNs in USA, a EPNs isolated of *Heterorhabditis* spp. was found from citrus root weevil, *Diaprepes* *abbreviates* and were identified as a new isolates. The commonly known species found to be *H. indica* and three isolates of *Steinernema* where two were newly undescribed.

Campos-Herrera *et al.* (2006) isolated a new isolates of *Steinernema feltiae* in La Rioja (Spain) from *Bibio hortulanus* larvae. A comparative morphometric evaluation of this new strain and other four additional isolates of *S. feltiae* were performed. Although the significant differences were observed in morphometric measurements, PCR, RFLP profiles and sequence analysis of the ITS region of rDNA through confirmed the identity of the new strain as A2 RFLP type of *S. feltiae.* A comparative morphometric studied was conducted among the EPNs from three different hosts such as *Galleria mellonella*, *Spodoptera littoralis* and *B. hortulanus*.

Shinde *et al.* (2008) conducted random surveyed in three tehsils of Banaskantha district of North Gujarat, *viz.*, Dantiwada, Deesa and Palanpur during 2002-2003 used soil baiting technique for isolation of EPNs. They recorded that all the EPNs isolated were *S. carpocapsae* and found only in one tehsil, Dantiwada.

Dubey *et al.* (2010) reported that out of 29 soil samples, one sample from rhizosphere of sapota in Agriculture Research Farm, Indira Gandhi Krishi Vishwavidyalaya, Koni, Bilaspur, revealed the presence of EPNs *i.e.* IARI-EPN-*chh*1 which was most closely related to *Steinernema carpocapsae* or might be representing a new isolates of *Steinernema* in Chhattisgarh.

Waliullah and Mantoo (2010) conducted random survey for collection of soil samples and isolation of EPNs where isolated the EPNs *viz.*, *H. indica* from grape orchards in Kashmir.

Razia and Sivaramakrishnan (2014) collected 200 samples in Kondikanal locality of Dindigul district of Tamil Nadu. Experiment conducted the larvae of *Galleria mellonella*, used as baiting technique for isolation of EPNs from seven samples whereas, three and five samples containing *Heterorhabditis* and *Steinernema* isolates respectively. The results of morphometric and molecular characterization data of isolated EPNs represented *Steinernema* spp. and identified as *S. siamkayai* which was isolated from three samples and one *Heterorhabditis* spp. *i.e. H. indica* respectively.

Kaushik and Chaubey (2016) identified a strain of EPNs from the cultivated lands in Meerut region of Western Uttar Pradesh. This strain was named CCS-EPN14S and showed maximum identity with *S. pakistanense* of 99.00 per cent and formed a highly supported clade with *S. pakistanense*. The base sequence (1-1205 bp) of this *Steinernema* isolates has been deposited in Gene Bank, NCBI, under the name of *Steinernema* spp. 14 Schhav vide accession no. KJ820773.

Sharma *et al.* (2016) isolated and identified EPNs from fruit orchards in Sirmour and Solan districts of Himachal Pradesh. Out of fifty six soil samples, eleven soil samples of 19.64 per cent were resulted to be positive for the isolated of EPNs collected from four different sites. Six samples of 10.71 per cent contained *Heterorhabditis* spp. which was identified as *Heterorhabditis bacteriophora*. Five samples of 8.93 per cent resulted *Steinernema* spp. which one was identified as a *S. feltiae*. One of the sites surveyed was found positive for both *Heterorhabditis* and *Steinerenema* spp.

Abirami *et al.* (2017) surveyed that the isolation of EPNs occurring in Tamil Nadu from three districts viz., Nilgiris Coimbatore and Erode and during September to December 2014. Total 126 soil samples were collected from three districts of Western Tamil Nadu were evaluated the presence of EPNs isolated from the two samples in two of the districts *viz.,* Coimbatore and Erode. First population was identified as *Steinernema abbas*i from Anamalais, Coimbatore and second population was identified as *S. carpocapsae* from Bhavanisagar, Erode.

## 2.2 To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh.

Danilov, (1976) stated that *Steinernema* spp. were active over a different range of temperatures and recorded that 25°C was the optimum temperature for growth and development. When temperatures increases of 32°C resulted quick mortality of *G. mellonella* larvae and normal mortality recorded at temperatures ranges from 10-32°C infected with *S. carpocapsae.*

Gaugler and Boush (1978) tested pathogenicity of *Steinernema feltiae* when IJs exposure to UV radiation (240 nm) for seven minutes on *G. mellonella* larvae and further, exposure for 2.50 to 5.00 minutes the development and reproduction were also inhibited.

Gaugler and Mulloy (1981) studied the effect of temperature on the host mortality and infectivity, which indicated that at a low temperature of 9°C, *S. carpocapsae* caused mortality of the host insects, but infection takes longer duration of 312 hours (14-15 days), where mortality of host insects larvae within 16 hours when the temperature was at 30°C.

The infectivity of *S. glaseri* and *Heterorhabditis* spp. 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).

Molyneux (1986) reported that suitable temperature for EPNs survival, infectivity and development for causing infection varies with EPNs strain. and species *S. carpocapsae* was found to be more effective at all the temperatures ranging from 10 to 28°C as compared to *S. feltiae* and *H. heliothidis.*

Westerman *et al.* (1989) studied that at 8 - 10°C under field conditions an isolated of *Heterorhabditis* species (Hf 85) could caused 100.00 per cent mortality of the *Otioryncus sulcatus*.

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

Georgis (1990) stated that the EPNs could be stored under refrigeration in aqueous suspension for increasing the shelf life under adequated supply of oxygen and temperature requirement which varies from species to species without affecting the bioefficacy.

Kaya, (1990) investigated the survival and pathogenicity of *S. carpocapsae* and *S. glaseri* in different types of soil. The survival per cent of *S. carpocapsae* was found to be 38.80, 44.90, 26.70 and 32.90 in sand, sandy loam, clay and clay loam soil respectively whereas *S. glaseri* survival per cent was to be 25.90, 30.10, 19.30 and 22.50 in sand, sandy loam, sand, clay and clay loam soil respectively.

Kung *et al.* (1990b) stated that the per cent survival of *S. carpocapsae* and *S. glaseri* was found to be lower in clay soil in comparison to clay loam and sandy loam. *S. carpocapsae* and *S. glaseri* was most active in sandy loam and sandy soil respectively.

Kung *et al.* (1990c) examined that survival of *S. carpocapsae* and *S. glaseri* at different levels of pH. Which observed gradually declined during 16 weeks as the tested pH, decreased from eight to four, survival rate of both species was reduced sharply after a week at pH ten. Survival per cent of both species was found to be similar at pH 8, 6 and 4 during started four weeks. Thus, the capacity of species varies at different pH levels. The per cent survival of *S. carpocapsae* was significantly greater than *S. glaseri* at duration of 16 weeks.

Barbercheck and Kaya (1991) conducted experiment to determine the effect of soil texture and host searching behaviour of *H. bacteriophora* and *S. carpocapsae* on larvae of *G. mellonella* in laboratory condition. Thus, resulted that *H. bacteriophora* was more mobile than *S. carpocapsae* in fine sandy loam and organic soils and least in clay soils.

Giffin *et al.* (1991) tested that the occurrence of *Heterorhabditis* which was highly correlated with ocean beaches of sand grains from coral and shell with at pH of 8 and also content low organic matter. Whereas, *Steinernema* isolates were found with high organic content in silty clay and silty loam soils (Hara *et al.*, 1991). High frequency of EPNs *i.e. Heterorhabditis* spp, *S. feltiae* and *S. affinae* from sandy and peat soils than clay and clay loam soils.

Kung *et al.* (1991) reported the effects of soil moisture, temperature and relative humidity on the per cent survival of EPNs *viz.*, *S. glaseri* and *S. carpocapsae* at the laboratory conditions in USA. Per cent survival of *S. carpocapsae* and *S. glaseri* was significantly higher at lower temperature of 5 to 25 and 5$^{o}$C than at the higher temperature of 35 $^{o}$C and 15 to 35 $^{o}$C respectively.

Zervos *et al.* (1991) studied the different inoculum doses of IJs of *H. heliothidis* and *S. glaseri* of 5.00, 10.00, 25.00, 50.00, 100.00 and 500.00 per larva of *G. mellonella* at different temperatures ranges of 5.00, 10.00, 15.00, 20.00, 25.00 and 30.00°C Thus, no IJs emergence was recorded at temperature 5.00 or 10.00°C. Development was quicker at 25°C in both the spp. No emergence of the *H. heliothidis* was observed at 30.00°C when inoculated with doses of 500.00 IJs per host. However, yield per host was greater at 20°C even with low level of inoculum of IJs.

Gaugler *et al.* (1992) tested the effect of UV radiation on EPNs per cent survival and resulted the complete inactivation of *H. bacteriophora* in 4.00 min. and in10.00 min. for *S. carpocapsae* and also tested pathogenicity of both the IJs against *G. mellonella* and resulted the loss of pathogenicity in *H. bacteriophora* found at 4.00 min. whereas, in 6.00 min. for *S. carpocapsae*.

Gouge *et al.* (1994) evaluated the infectivity of *G. mellonella* by *S. carpocapsae* and *S. feltiae* between 19.20 and 6.40 °C after storage of IJs. Both EPNs infected the host when temperature is lower at 13.79 and 11.00°C respectively. Therefore, *S. carpocapsae* resulted no host mortality was found at below 15.92°C. The infectivity of *S. carpocapsae* was significantly higher at 20°C when the EPNs has stored at lower temperature of 6.40°C.

Nickel and Shapiro (1994) reported that Blankophor and Tinopal were the most effective UV screens for *S. carpocapsae* resulted 95.00 per cent infectivity of the larvae of *G. mellonella* at after four hrs. of exposure to UV radiation. They recorded per cent infectivity of 85.00 and 80.00 preserved by DML and Blankophor HRS and also preserved 70.00 per cent infectivity by P 167 after the four hrs. of exposure to UV radiation.

Ghally (1995) examined that the activity of *H. heliothidis* and *S. carpocapsae* at different ranges of temperature and found optimum soil temperature at 20-25°C was suitable for *H. heliothidis* and *S. carpocapsae* caused infection on cotton leaf worm, *Spodoptera littoralis.*

Williams and Macdonald (1995) stated that *Heterohabditis* spp. and *S. feltiae* caused per cent mortality and significantly reduction of number of leaf miner larvae *viz., Liriomyza huidobrensis* at temperature ranges from 10 to 30°C.

Hsiao and Hsiao (1996) studied the movement of *S. carpocapsae* in different types of soils and resulted more movement observed in sand and silty loam soils than in coarse sandy loam and sandy clay loam soils. When IJs were placed on the top of the soil surface, then least downward movement were observed at temperatures of 20.00, 25.00 and 35.00°C in all types of soil. At high temperature of 35°C no EPNs were found from 6.00 cm depths. Therefore, the higher numbers of EPNs were recovered from silty loam and sandy clay loam soils at 5.00 cm and 10.00 cm depths which indicated the migration of IJs tends to decrease as increases the proportions of silt and clay in soil.

Shamseldean *et al.* (1996) reported the effect of soil temperature on efficacy of four species of EPNs caused infection and mortality of *Spodoptera littoralis* in aspects to soil temperature, IJs doses and emergence from the cadavers in laboratory condition. Results revealed that all the tested IJs of *H. bacteriophora* caused 100.00 per cent mortality of *S. littoralis* at 4.00, 10.00 and 25.00°C and least mortality recorded at 35°C.

Cheng and Hou (1997) studied that all EPNs *i.e*. *Steinernema* and *Heterorhabditis* spp. died continuously after exposure to UV radiation (254 nm) for 36 hours and incubated at pH of 4.00 to12.00 in phosphate buffer for 10 days, the per cent survival of IJs about 60.00 to 70.00, and at low pH of 2.00, all the IJs were found to be dead.

Lacey and Unruh (1998) conducted the experiment for tested the effect of temperature at different ranges *viz.*, 15.00, 20.00, 25.00 and 30.00°C on the susceptibility of larvae of the codling moth, *cydia pomonella* to *S. riobrave, S. carpocapsae* and *H. bacteriophora* and resulted that *S. carpocapsae* caused highest host mortality ranging from 66.00 to 90.00 per cent at 25.00°C than any other EPNs.

Karunakar *et al.* (1999) conducted the experiment of effect of different ranges of temperature *viz.*, 10.00, 12.50, 15.00, 25.00, 27.50, 30.00, 32.50 and 35.00°C on infection, penetration and multiplication of *S. glaseri, S. feltiae and H. indicus* by using the larvae of *G. mellonella.* EPNs infectivity and penetration on *G. mellonella* was recorded from 12.50 to 32.50°C, multiplication was recorded from 25.00 to 32.50°C. No host mortality was recorded at 10.00 and 35.00°C *Steinernema* spp. showed higher penetration into the host at 25°C, while *H. indicus* at 27.5°C. Mass multiplication was significantly higher in *H. indicus* followed by *S. feltiae* and *S. glaseri.*

Hussaini *et al.* (2000) analyzed the performance of four EPNs species were found to be better in sandy loam soil than in sandy soil at 5.00 cm depthS against *Agrotis ipsiolon*. Combination of *S. carpocapsae* and *H. indica* had an additive effect over their individual populations in both types of soils at 10 cm depths.

Strauch *et al.* (2000) examined the per cent survival of EPNs as dauer juveniles of *H. bacteriophora* and *H. indica* at temperatures ranges between 5.00 and 25.00 $^{o}$C in Germany and recorded that a maximum per cent survival of *H. indica* was achieved at 15.00 $^{o}$C and *H. bacteriophora* survived best at 7.50 $^{o}$C and least at 25.00 $^{o}$C.

Elawad, *et al*. (2001) evaluated progeny production of a new EPNs *viz., Steinernema abbasi* in various lepidopterans larvae. Most of IJs developed in the *G. mellonella* 2,34,000 IJs, but the in Cotton bollworm *Helicoverpa virescens* 2,20,000 IJs and in *Spodoptera exigua* 1,66,000 IJs were recorded. Progeny production was number of IJs invading to the host in between the doses of 40 and 230 IJs. The infectivity declined due to storage of IJs for 3 months, but the IJs were still infective approximately 11 per cent at 30°C.

Hazir *et al*. (2001) tested the EPNs isolates of *S. feltiae* at different temperature ranges. The isolates were exposed to 5.00, 8.00, 10.00, 15.00, 20.00, 25.00, and 28.00°C on larvae of *G. mellonella*, the data of mortality and progeny production were recorded. All isolates caused mortality per cent of 100.00 of greater wax moth larvae and development and progeny production in between 8 and 25°C. At 28°C, mortality was 100.00 per cent, but no progeny was recorded. The highest IJs progeny was recorded at 15.00°C for all isolates. At 25°C, the IJs of *S. feltiae* isolates emerged from from 5 to 7 days in the cadavers. The efficiency of *S. feltiae* for penetration of hosts at 5.00, 8.00, and 10.00°C. Penetration of IJs was consistently higher for all isolates at 15.00, 20.00, 25.00 and 28.00°C no progeny production was observed at 28.00°C.

Hussaini and Sankaranarayanan (2001) reported mortality of *G. mellonella* in sandy and sandy loam soils with *Steinernema* spp. and *Heterorhabditis* spp. at 5.00 and 10.00 cm depths. Thus, the infectivity of *Steinernema* spp. was higher at 5.00 cm depth and progeny was recorded higher at sandy loam soils.

Chen *et al.* (2003) studied that host mortality was significantly affected by EPNs species. For all species, mortality was higher at 20.00°C than at 15.00 and 10.00°C. *S. feltiae* was the only that killed the final instar larvae of *Delia radicum* at different temperatures within both two and four days. *S. arenarium*, *S. carpocapsae* and *H. megidis* were to kill the larvae within two days at 15°C and 20°C, whereas *H.*

*bacteriophora* was effective only at 20°C. At all temperatures *S. feltiae* showed the higher virulence than *H. bacteriophora*.

Banu and Rajendran (2003) tested the effect of UV radiation on survival of EPNs and recorded the absolute mortality of the IJs of *S. glaseri, H. indica* with continuously exposure to UV radiation for 120.00 to 135.00 min.

Koppenhoffer and Fuzy (2003) stated the ecological characteristics of EPNs, *viz.*, *Steinernema scarabaei* that were originally isolated from epizootics in scarab populations from turfgrass areas in New Jersey. *S. scarabaei* infected a limited range of hosts and appeared as best adapted to scarab larvae as hosts in laboratory conditions. It used foraging techniques with a low attachment rate to mobile hosts on the soil surface but with caused infection on sedentary host placed at $P_2$ cm depth of soil. The optimum infectivity recorded from 17.50 to 25.00$^{o}$C. *S. scaraba*ei has good potential for the control of scarab pests.

Subramanian and Senthamizh (2004) tested the effect of UV radiation on IJs of *S. glaseri* and *H. indica.* The results revealed that UV light emitted from UV lamp of 15W caused 100.00 per cent mortality of *S. glaseri* and *H. indica* after 210.00 and 120.00 min. of continuously exposure respectively.

Campos-Herrera *et al* (2006) reported a new strain of *Steinernema feltiae* isolated in La Rioja (Spain) from larvae of *Bibio hortulanus*. Ecological characterization of the Rioja isolates was performed in larvae of *G. mellonella*. Larval mortality per cent was 75.30 and 78.12 in penetration and sand column assays respectively, and the per cent penetration of IJs was 12.00 and 2.80 in these assays. Larval mortality in the one-on-one bioassay was 4.20 and in exposure of time bioassays, it was 50.00 per cent at 11.25 hours.

Morton and Fernando (2009) isolated five isolates of *Steinernema* and two isolates of *Heterorhabditis* species to hypoxia. Survival of isolates differed significantly among the tested EPNs with duration of exposure. After 24 hours of exposure the EPNs survival rate varied from per cent of 11.40 to 100.00. The *S. feltiae* strain showed the higher tolerance than the rest of the isolates. Survival of IJs decreased with duration of all the isolates tested. After 96 hours of exposure to hypoxic conditions, survival ranged from per cent of 59.10 to 0.00. *H. bacteriophora* strains and *S. carpocapsae* indicated low to moderate survival of IJs..

Sunanda (2009) studied the effect of temperature on the life cycle of *S. abbasi* and *H. indica* which resulted that IJs were able to penetrate *G. mellonella* at ranges of temperature between 20.00$^{o}$C and 30.00$^{o}$C for both the species. The maximum number of IJs of *S. abbasi* and *H. indica* emerged from *G. mellonella* larvae at 30.00$^{o}$C and 25.00$^{o}$C.

Radova and Trnkova (2010) analyzed the impact of soil temperature on the virulence of the EPNs such as *S. carpocapsae* and *S. feltiae*. The effect of temperature of 10.00, 15.00, and 25.00 $^{o}$C tested against the larvae of *Tenebrio molitor*. The EPNs were tested at two concentrations levels of 50.00 and 500.00 IJs per box. *S. carpocapsae* was significantly more efficient at the highest temperature of 25.00 $^{o}$C than *S. feltiae*, at the lower concentration of 50.00 IJs per box. *S. feltiae* recorded higher host mortality at lower temperatures of 15.00 and 10.00 $^{o}$C.

Rohde *et al* (2010) examined the effect of soil temperature and moisture on the infectivity of *Heterorhabditis* spp. and *Steinernema carpocapsae.* All third instars larvae of *C. capitata*, and to compare the efficiency of these isolates at five different soil temperatures of 19.00, 22.00, 25.00, 28.00, and 31.00 °C and three levels of relative soil moisture such as 100.00, 75.00, and 50.00 per cent of field capacity. Mortality evaluated after five days when observed the infection symptoms and cadaver dissection. The infectivity was directly proportional to temperature increase, with maximum percent mortality per cent of 86.70 and 80.00 for *S. carpocapsae* and *Heterorhabditis* spp., respectively, at 31.00°C. At 25.00°C, the highest mortality for both species was obtained atper cent of 75.00 of field capacity at per cent of 96.70 and 26.70 for *S. carpocapsae* and *Heterorhabditis* spp. respectively.

Andalo *et al*. (2011) studied that the lipid reserves were conserved for longer storage periods at 8.00, 16.00, and 20.00$^{o}$C, while at 24.00 and 28.00 $^{o}$C the per cent of lipids decreased rapidly. The infectivity of IJs of *Heterorhabditis* spp. was lesser tolerant than those of *steinernema* spp. to temperature of 8.00, 16.00, and 20.00 $^{o}$C.

Raja *et al.* (2011) reported the ecological characterization of *Steinernema siamkayai* Tiruchirappalli strain from India. The effect of temperature on IJs infectivity, development and foraging behaviour were determined. The data recorded that *S. siamkayai* was a warm-adapted EPNs species with larval mortality between 15.00$^{o}$C and 37.50 $^{o}$C and IJs progeny production in between 20.00 $^{o}$C and 35.00 $^{o}$C. Larval mortality of *G. mellonella* by *S. siamkayai* on different substrates such as sand, filter paper, filter paper sprinkled with sand was 100.00 per cent on all substrates. Number of IJs out of 100 IJs that penetrated into *G. mellonella* host at different soil depths was the highest at the surface 44 IJs per larva and the lowest at 5 cm depth 13 IJs per larva and no larval mortality recorded at 10 cm depth.

Mejia-Torres and Saenz (2013) isolated the EPNs *Heterorhabditis* sp. from soil in Alcalá, Valle del Cauca (Colombia). The effect of temperature on the infectivity viability and reproduction were evaluated in IJs. The significant differences were found in the infectivity viability and reproduction of the IJs at different temperatures. No nematodes were recorded at 5.00 °C and 10.00 °C, and 35.00 °C also, and at 25.00 °C best for both survival and infectivity.

Shapiro-ilan *et al*. (2014) reported two strains of the EPNs, *Heterorhabditis floridensis (*332 in Florida and K22 isolated in Georgia) were described. The *H. floridensis* strains caused higher mortality or infection in *G. mellonella* at 30.8 $^{o}$C and 35.8 $^{o}$C compared with *S. riobrave* 35.5 $^{o}$C, a strain known to be heat tolerant, and the *H. floridensis* strains were also capable of infecting at 17.8 $^{o}$C where as *S. riobrave* at 35.5 $^{o}$C was not. However, at higher temperature of 37.8$^{o}$C and 39.8$^{o}$C through *H. floridensis* infected *G. mellonella*, *S. riobrave* strains also caused higher mortality.

Pervez *et al*. (2015) conducted experiment where attachment, penetration, infectivity and multiplication of native isolates of EPNs *viz.,* one isolates of *Heterorhabditis* spp. three isolates of *Steinernema* spp. one isolates of *Oscheius* spp. and one isolates of *O. gingeri* were tested against larvae of *Conogethes punctiferalis* at different temperatures *viz.*, 20.00, 25.00, 30.00 and 35.00°C. Among all the temperatures, maximum mortality of host was found at 30.00°C followed by 25.00°C, whereas the least mortality was recorded at 20.00°C and 35.00°C. Maximum number of IJs was multiplied at 30.00°C and minimum was recorded at 35.00 °C. Among the entire tested EPNs, no multiplication of *Heterorhabditis, Steinernema* sp. and *O. gingeri* was recorded at 20°C.

Therefore, the optimal temperature for infection and development for all promising EPNs was 30.00°C.

Raheel *et al.* (2015) examined the infectivity of four EPNs species *i.e. Heterorhabditis bacteriophora*, *H. indica, Steinernema feltiae* and *S. asiaticum* in different soil textures such as loamy sand, sandy loam and clay loam. The *in-vitro* assessment of the infectivity was done by exposing final larval instar of *G. mellonella* to EPNs. Thus, resulted the infectivity was greater in sandy loam soil 71.42 per cent followed by clay loam 54.75 per cent, while it was lower in loamy sand 41.63 per cent. Among all species tested, *H. bacteriophora* showed maximum infectivity of 69.82 per cent, followed by *H. indica* 52.36 per cent, *S. feltiae* 52.36 per cent and *S. asiaticum* 49.19 per cent showing similar trends.

Sharmila and Subramanian (2016) stated the survival and infectivity of EPNs at different storage temperatures. The EPNs *Heterorhabditis indica* and *Steinernema glaseri* differed in their survival and causing infection on hosts at different ranges of temperatures for storage. The best storage temperatures for survival of *H. indica* were recorded 20 .00 and 25.00 $^{o}$C under BOD conditions for 90 days. Also, the infectivity was upto 90 days at different ranges of temperatures for storage of 10.00, 20.00 and 25.00 $^{o}$C were tested against *Corcyra cephalonica, S. glaseri* survived longer upto 120 days and per cent survival of 100.00 were recorded at the temperature ranges of 10.00, 20.00 and 25.00 $^{o}$C and the infectivity was upto 60 days at 20.00 $^{o}$C were tested against *Corcyra cephalonica.*

Raheel *et al.* (2017) reported that the influence of different ranges of temperature on reproductive potential of native and exotic species of EPNs was tested on *Galleria mellonella* larvae. The native species included *Steinernema asiaticum* and *Heterorhabditis indica* whereas exotic species were *S. feltiae* and *H. bacteriophora*. 300 IJs were exposed on *G. mellonella* larvae of each species. After inoculation at different temperatures, the reproductive potential of EPNs increased with increasing temperature and was recorded to be the best at 25$^{o}$C. No EPNs species recovered at 5$^{o}$C. *S. feltiae* started progeny production at 10$^{o}$C, while all remaining species recovered at 15$^{o}$C or higher temperature. More numbers of IJs were recovered from *H. bacteriophora*. Time taken for first emergence of IJs from the host cadaver was quickest at 25 $^{o}$C within 7-8 days in case of *S. asiaticum* and *S. feltiae*, while 11-13 days for *H. bacteriophora* and *H. indica*. Maximum time of emergence was taken by *S. feltiae* at 10 $^{o}$C.

**2.3 To study the mass multiplication of EPNs at laboratory condition on different hosts.**

Glazer and Navon (1990) studied the pathogenicity of EPNs *viz.*, *Steinernema* and *Heterorhabditis* on *H. armigera* recorded the complete mortality with inoculum of 200.00 IJs of *S. feltiae* filipjev, strain and 54.00 IJs was observed per host insect was $LD_{50}$ and the duration of eight hours of hosts exposed to EPNs resulted mortality more than 80.00 per cent.

Cabanillas and Raulston (1992) conducted experiment for studied the efficacy of *Steinernema riobravis* in *Helicoverpa zea* where the EPNs inoculum two billion IJs per larva and pupa of *H. zea* applied to soil and observed 95.00 to 100.00 per cent infection in both larva and pupa.

Karunakar *et al.* (1992) mass multiplied *Steinernema glaseri S. feltiae,* and *Heterohabditis bacteriophora* on *Scirpophaga excerptalis* and *Chilo sacchariphagus indicus* where progeny produced upto 3,70,000 IJs of *S. glaseri,* 1,27,000 IJs of *S. feltiae,* and 2,10,000 IJs of *H. indica* from final instar larvae of *Chilo saccariphagus indicus* of inoculum of 20.00 IJs per larva.

Singh (1993) tested cross infection of EPNs of *Steinernema* spp. isolated from *Agrotis segetum* cadaver used against second instar larvae of *Papilio demoleus* and mortality observed 45.00 per cent within 48 hours on *P. demoleus*. Whereas, further mortality observed at per cent of 10.00 to 50.00 from the surviving larvae of later five days. After inoculation, cumulative mortality recorded of per cent 90.00 to 100.00 within next 10 days.

Otto (1995) inoculated the EPNs *viz., Steinernema riobravis*, *S. carpocapsae* and *Heterorhabditis* spp. on *Helicoverpa armigera*. He recorded that the relationship between the doses of IJs and the no. of EPNs established which, are directly proportional to each other. *H. armigera* was to be found more susceptible to *S. riobravisi* than any others larvae.

Zaki *et al.* (2000) reared silk worm larvae, *Bombyx mori* for mass multiplication of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, where an average progeny harvest from third instar larvae of 48,703.00 and 2,750.00 IJs of *S. carpocapsae* and *H. bacteriophora* respectively. Thus, no EPNs could be established from the final instar larvae of silk worm.

Anonymous (2001). Progeny of *H. bacteriopora* could produce 3.56 lakhs IJs per larva from *Spodoptera litura* and 3.16 lakhs IJs per larva from *Heliothis armigera*. Among all lepidopteran larvae tested progeny of *H. bacteriophora* could be produced 13.3 per cent was higher in *H. armigera* larvae than other *Opisina arenosella* and *Plutella xylostella* larvae.

Singh *et al.* (2001) tested the efficacy of *H. bacteriophora* against white grubs after the exposure of 4 days. The $LD_{50}$ values for first, second and third instars larvae were 110, 326 and 989 IJs per grub, respectively by using filter paper impregnation method and the $LD_{50}$ values were comparetively higher as 1875, 5097 and 8942 IJs per grub, respectively through using soil inoculation method.

Anonymous (2002), the optimum level of IJs varies from 50.00 to 75.00 IJs required for causing infectivity. The highest progeny production recorded in *G. mellonella* as compared to any other hosts insects like *Agrotis ipsilon* which were used as mass rearing of EPNs. Thus, the factor is common for both the EPNs *viz.*, *Steinernema* and *Heterorhabditis*, whereas, *S. carpocapsae* resulted highest yield at inoculum levels of 50.00 IJs and *H.indica* at inoculum of 75.00 IJs.

Rajkumar *et al.* (2002) conducted experiment on culturing of EPNs on different size and weight of larvae in Udaipur. Progeny production of EPNs on three different body size and weight of larvae on *G. mellonella*. In large sized larvae (20-25 mm) highest progeny recorded of *Steinernema* spp. harvested 90,945.00 IJs per larva and in case of *Heterorhabditis* spp. harvested 2,01,520 IJs per larva.

Gupta (2003) reared *Steinernema carpocapsae* on fourth instar larvae of *Corcyra cephalonica* through *in-vivo* method. *S. carpocopsae* inoculated 20.00 IJs per larva and were kept at 20 – 24°C for incubation period. After emergence of IJs, were harvested from the cadavers after 10-12 days.

Shapiro-Ilan *et al.* (2004) reported that the EPNs *viz.*, *Steinernema* and *Heterorhabditis* kills host insects with the help of symbiotic bacteria. These nematode-bacteria complex were mass-multiplied for biopesticides in both *in-vivo* or *in-vitro* methods, *i.e.* solid or liquid fermentation. *In-vivo* production has low technology with low costs and resulting high qualities also, *in-vivo* production and solid culture may be improved through innovation in mechanization and streamlining.

Chandel *et al.* (2005) conducted experiment in which mixing of *H. indica* with FYM during first week of July in potato crops at Kufri in Shimla hills. There was 8.13 per cent tuber infestation due to *Brahmina coriacea* white grubs in treated plots as compared to 11.28 per cent in control plots. Thus, under the laboratory conditions, they observed percent mortality of 100.00 of second instar grubs of *B. corecea* after 28 days after inoculation. In third instar grubs, per cent mortality of 80.76 was recorded.

Prabhuraj *et al.* (2006) stated that mortality of larvae of *H. armigera* was directly proportional to increased doses of *H. indica.* They observed that third instar larve of *H. armigera* was more susceptible than fourth instar. There was complete mortality of third instar larvae 100.00 IJs per larva, whereas in fourth instar, 94.70 per cent mortality was observed.

Singh and Gupta (2006) reported the occurrence of EPNs in Himachal Pradesh and tested their pathogenicity against broad range of host insects belonging to four orders. *H. bacteriophora* was found to be more virulence than either *S. feltiae* or *Sterinernema* spp. The whitegrub, *B. corecea* and greasy cutworm, *A. ipsilon* were more susceptible to these IJs.

Saravanapriya and Subramanian (2007) examined that *H. indica* and *S. glaseri* against *H. armigera, S. litura, P. xylostella* and *Cnaphalocrosis medinalis* in Tamil Nadu. The larvae of *P. xylostella* were found to be more susceptible to both these EPNs with $LC_{50}$ values of 2.01 and 2.53 IJs per larva, followed by *C. medinalis* $LC_{50}$ values of 5.46 and 5.17 IJs per larva, *S. litura* $LC_{50}$ values of 7.32 and 9.04 IJs per larva and *H. armigera* $LC_{50}$ values of 9.40 and 10.51 IJs per larva. For pupal stage, the $LC_{50}$ values of *H. indica* were 73.29, 86.28, 104.45 and 120.79 IJs per pupa for *S. litura, P. xylostella, H. armigera* and *C. medinalis,* respectively. The $LC_{50}$ values of *S. glaseri* for pupal stages of

*P. xylostella, C. medinalis, S. litura* and *H. armigera* are reported to be 95.29, 161.67, 108.12 and 122.73 IJs per pupa, respectively

Nyasani *et al.* (2007) rported that EPNs have a great potential against diamondback moth larvae management in Kenya. They evaluated five different Kenyen EPNs *viz., S. karii, H. indica, S. waiseri, Heterorhabditis* spp. and *Steinernema* spp. The $ET_{50}$ (Exposure time 50) values of tested EPNs ranged from 20.27 to 38.12 hours and it was significantly higher for *S. karii* than of *H. indica.*

Gupta *et al.* (2008) tested local isolate of *S. carpocapsae* in Kashmir which recorded 100.00 per cent mortlity of third to fifth instar larvae of *S. litura* after 96 hours of treatment in laboratory. Inoculum of 160 IJs per larva on fifth instar larvae of *S. litura* produced maximum IJs of 3.29 x $10^5$. The $LC_{50}$ values varied between 11.41 and 27.17 IJs per larva in all the instars.

Lalramliana and Yadav (2009) examined *H. indica, S. thermophilum* and *S. glaseri* at doses of 10.00 to 100.00 IJs per larva against *P. Brassicae* in Shillong. The $LC_{50}$ values of 30.20 IJs per larva, *S. feltiae* was found to be highly effective. However, progeny production of *H. indica* was higher on larvae of *P. brassicae*.

Chandel *et al*. (2009) evaluated the potential of *H. bacteriophora* in Himachal Pradesh against *A. segetum*. The testing was done against third, fourth and fifth instar larvae at doses of 10.00 to 40.00 IJs on petri plates and at 1000.00 to 5000.00 IJs/ kg soil. In soil, 1000.00 IJs/kg were sufficient to cause infection and mortality per cent upto 61.30 on fifth instar larvae. There was increase in larval mortality with increase in exposure of time and as the aged increased, the mortality decreased.

Fetoh *et al.* (2009) reported that *S. carpocapsae and H. bacteriophora* used against *A. ipsilon* under laboratory and field conditions. Both these species of EPNs were found to be more effective at 100.00 IJs as compared to 25.00 IJs per larva which clearly indicated that with increase in doses of IJs, the mortality increased.

Sankar *et al.* (2009) studied the pathogenicity of *H. indica* against *G. mellonella* and tested its compatability with other biopesticides. The combination of *Pseudomonas fluorescence* with *H. indica* was most efficient causing 100.00 per cent mortality on *G.*

*mellonella* after 24 hours of storage. Progeny produced by *H. indica* on *G. mellonella* was maximum 1,40,108 IJs per larva in the combination treatment with *Trichoderma viridae.* Pathogenicity of *H. indica* increased when exposed with other biopesticides on host larva proved to be more virulent and compatible.

Divya *et al*. (2010) evaluated the pathogenicity of *H. indica* against larval of *H. armigera, S. litura and G. mellonella.* All the stages of larvae of tested host insects were more susceptible to *H. indica*. However, the degree of susceptibility differed from the instars with doses and periods of exposure. In the dose responsed to bioassay, second and third instar larvae were found to be more susceptible at 300.00 IJs of *H. indica* per larva than fourth and fifth instar larvae exposed for 24 hours. Under greenhouse conditions, per cent larval mortality of *H. armigera* and *S. litura* with *H. indica* at 25.00 ml per plant was significantly more on final instar larvae per cent of 62.87 and 56.75 respectively, after 60 hours of treatment.

Adiroubane *et al.* (2010) tested the efficacy of *S. siamkayai* against *S. litura, P. xylostella*, *Leucinodes orbonalis*, *Earais vitella* and *C. medinalis* which were isolated from Karaikal region of Puducherry. There was increase in susceptibility with an increase in duration of exposure.

Hyrsl (2011) stated that *H. bacteriophora, S. glaseri, S. scarabaei and S. feltiaeare* were broadly used against host insect pests of commercial crops. Pathogenicity studied against *G. mellonella* resulted that IJs of EPNs killed host within 48 hours, and mortality of host insect was correlated with number of invaded IJs. The invasion process is very fast, with entering of IJs on insect host within a few hours.

Kumar and Ganguly (2011) examined *S. thermophilum* (New Delhi strain), *S. meghalayensis* (Meghalaya strain), *S. riobrave* (Gujarat strain), *S. harryi* (Tamil Nadu strain) against third instar nymphs of solenopsis mealybug (*Phenacoccus solenopsis*), adult cotton aphid (*Aphis gossypii*) and second instar nymphs of cotton whitefly (*Bemisia tabaci* at 50.00 and 500.00 IJs/ml in sand well and leaf disc assays. *S. thermophilum* caused 83.00 per cent mortality of mealybugs within 72 hours after inoculation at 50.00 IJs per ml and 100.00 per cent within 48 hours at 500.00 IJs per ml against aphid, *S. thermophilum* caused 66.00 and 83.00 per cent mortality at 50.00 and 500.00 IJs per ml, respectively within three days of treatment. All tested *Steinernema* spp. were ineffective against whitefly at 50.00 IJs per ml, however, at 50.00 IJs per ml, *S. riobrave* recorded 66.00 per cent mortality within 72 hours after inoculation. *S. meghalyensis* was the least effective strain.

Prasad *et al.* (2012) examined Meerut- strain of *H. indica* using filter paper and soil column assay against different lepidopteran and coleopteran pests. The larvae of *P. xylostella, H. armigera, L. orbonalis* and *Earais vitella* were highly susceptible to *H. indica* causing 73.00 to100.00 per cent mortality after 48 hours of exposure. The $LC_{50}$ values were calculated to be 132.00, 290.00, 475.00 and 810.00 IJs per larva of *P. xylostella, H. armigera, L. orbonalis* and *E. vitella*, respectively and against *S. litura, Spilosoma obliqua, P. brassicae* and *H. consanguinea* low susceptibility was recorded.

Kepenekci *et al*. (2013) tested efficiency of three Turkish isolates of the EPNs such as *Steinernema carpocapsae, S. feltiae* and *Heterorhabditis bacteriophora* were used against the last instar of potato tuber moth (PTM) *Phthorimaea operculella* under laboratory conditions. To determine the optimum IJs application rate and temperature, the experiments were conducted with 100.00, 500.00 and 1000.00 IJs at 10.00, 15.00 and 25.00$^{o}$C. Temperature and IJs concentration had a significant effect on *P. operculella* larval mortality. *S. carpocapsae* and *H. bacteriophora* species displayed generally increased virulence in parallel with rising temperature and the number IJs applied. At 25.00°C and 1000.00 IJs concentration, the larval mortality per cent was 96.00 and 80.00 for *S. carpocapsae* and *H. bacteriophora,* respectively. However, *S. feltiae* did not exhibit more than 40.00 per cent mortality at any temperature or concentration, except when the IJs were applied in infected insect host cadavers. At 25°C, infected cadaver applications showed 97.00, 83.00 and 67.00 per cent mortality for *S. carpocapsae, H. bacteriophora* and *S. feltiae*, respectively. Thus, results indicate that *P. operculella* larvae are quite susceptible to EPNs infection of *S. carpocapsae* black sea strain has a high potential to control this pest.

Rishi and Prasad (2013) examined efficacy of an indigenous EPNs, *H. indica* against lepidopteran insect pests, in which the larvae of *Plutella xylostella, Leucinodes orbonalis, Corcyra cephalonica, Galleria mellonella* and *Helicoverpa armigera* were found to be highly susceptible to the IJs of *H. indica* causing mortality from 73.30 to 100.00 per cent after 48 hours of exposure while, *S. litura*, *Spilosoma obliqua, Pieris brassicae,* and *Earias vittella* were less susceptible with a mortality ranging from 3.30 to 73.50 per cent.

Sharifi *et al*. (2014) reported that the efficacy of two species of EPNs *viz.*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* against last instar larvae of longhorned beetle, *Osphranteria coerulescens* were tested under laboratory conditions. In plate assays, the larvae were susceptible to both EPNs species but were more susceptible to *S. carpocapsae* of per cent mortality of 65.00 to 97.50 than *H. bacteriophora* of 42.50 to 87.80 per cent. Both EPNs species were able to penetrate and reproduce within *O. coerulescens* larvae, but production rate for *H. bacteriophora* was higher than those of *S. carpocapsae*; however, the penetration rate for *S. carpocapsae* was greater than *H. bacteriophora*. In a migration test on agar plate, *S. carpocapsae* showed negligible attraction to the pest or *Galleria mellonella*.

Tess *et al.* (2016) mass multiplied of EPNs *viz.*, *Heterorhabditis bacteriophora* as a bio-control agent using as symbiotic bacteria *Photorhabdus luminescens* on a solid media surface. The process of growing these EPNs was the surface area of a solid agar media, thus increasing the yield of the EPNs. The solid agar media was adjusted to conditions, which provided an ideal growing condition for these EPNs to maintain viability for an entire life cycle. The bacterial symbiont was then inoculated by an *in-vitro* culture 24 hours prior to IJs inoculation and further more leading to the inoculation of *Heterorhabditis bacteriophora*. The inoculated EPNs have a 7 to 8 day life cycle. Once the EPNs develop into IJs, the hermaphrodites can begin to self-fertilize its eggs and to produce new offspring.

Zolfagharian *et al.* (2016) reported that the susceptibility of *P. xylostella* larvae against two species of EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* was examined under laboratory conditions. Leaf bioassays were conducted to evaluate the IJs capability to reach the larvae and kill them. High larval mortality per cent of 72.60 to 96.00 was observed in laboratory. The $ET_{50}$ of *H. bacteriophora* was higher than that of *S. carpocapsae*. The $ET_{50}$ of EPNs such as *H. bacteriophora* and *S.*

*carpocapsae* tested ranged from 21.00 to 139.70 and 11.30 to 71.4 hours, respectively. The effect of both factors IJs and exposure time $ET_{50}$ of 50.00 per cent on the larval mortality respectively. Thus, EPNs have great potential against diamondback moth, *P. xylostella* management.

Rahoo *et al.* (2017) conducted mass production techniques in Pakistan, the development and application of EPNs depend on host insects for *in-vivo* production. As there is little information regarding relationship between IJs dosage and production of IJs of *Steinernema feltiae* and ability to locate hosts, therefore, in the present investigations were done on these aspects. A significantly greater emergence of IJs of *S. feltiae* from *G. mellonella* was observed with White traps than with modified Baermann trays. Maximum IJs of *S. feltiae* emerged at 50.00 IJs doses followed by 100.00 IJs. The emergence of IJs decreased significantly at the doses of 200.00 IJs and then increased with 400.0 IJs doses. The minimum number of IJs emerged at the doses of 200.00 IJs. The relationship between inoculation doses and emergence of IJs by using both methods was non-significant. Similarly, with an increase in inoculum doses and time, invasion of host was significantly increased. There was little invasion at the 800.00 IJs doses even after 4 days whereas with the commercial doses of 8,000 IJs did migrate to the larvae and caused some infection after 2, 3 and 4 days. Greatest invasion took place with the highest doses (80,000) with 8 IJs successfully finding and penetrating the larvae. A positive correlation was observed between doses and time and invasion of the host. It is concluded that application of EPN in cadavers may be appropriate in Pakistan because of the non-availability of industrially produced isolates.

Sankaranarayanan *et al.* (2017) evaluated two native EPNs isolates of, *Heterorhabditis indica* viz., DSM78 and BNR against white grub *Holotrichia serrata* in sugarcane were conducted in two separate field trials. In field trial I, three to four days-old *H. indica* (BNR) infected cadavers of *Galleria mellonella* were applied manually near the root zone at five inch depth. In field trial II, talc formulated *H. indica* (DSM78) was applied @ 4 billion IJs/ac. Field efficacy of EPN, *H. indica* (BNR) against white grub in sugarcane in the field trial I revealed considerable reduction of white grub population due to EPN application compared to initial grub population. About 51% reduction of grub population was reported at 15 days after EPN inoculation and increasing trend (87.3%) of grub mortality was observed at 30 days after EPN inoculation. In field trial II, *H. indica* (DSM78) treated field recorded 46.6% control of white grub population compared to the initial grub population.

Rahoo *et al*. (2018) stated that the suitability of EPNs as biological control agents of specific target insects is affected by their level of infectivity and reproductive capacity. Therefore, in the present study the productivity of five EPNs *viz., Steinernema feltiae, S. kraussei, S. carpocapsae, Heterorhabditis bacteriophora* and *H. indica* were compared in *Galleria mellonella* larvae. The production of IJs in *G. mellonella* was significantly affected by EPNs species. Significantly higher numbers of IJs were produced by *Heterorhabditis* species than *Steinernema* species in the cadaver. The production of IJs was the maximum in the case of *H. bacteriophora* which was not statistically different form *H. indica*. Minimum IJs were produced by *S. feltiae*. The IJs produced by *S. kraussei* and *S. carpocapsae* were statistically similar. The emergence of *Steinernema* spp. started from the 14$^{th}$ day and that of *Heterorhabditis* spp. from the 17$^{th}$ day. In case of *Heterorhabditis*, the maximum emergence of *H. bacteriophora* IJs (199,894) was recorded on the 23$^{rd}$ day and that of *H. indica* on the 20$^{th}$ day (99,495). On the other hand, in case of *Steinernema,* the maximum emergence of IJs of *S. feltiae* and *S. kraussei* was recorded on the 17$^{th}$ day (36,180 and 45,225 respectively) and that of *S. carpocapsae* on the 20$^{th}$ day (21,407). It is concluded that there was greater emergence of IJs from the *Heterorhabditis* species than those from the *Steinernema* species and hence can be used for the management of insect pests.

# CHAPTER – III

# MATERIALS AND METHODS

The present investigation entitled **"Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh"** was carried out in the Biological control laboratory, Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the year 2017- 18 with the following objectives:-

**Objectives:-**

1. To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.
2. To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh.
3. To study the mass multiplication of EPNs at laboratory condition on different hosts.

**3.1 Geographical location:**

Raipur is situated in the central part of Chhattisgarh and lies at 21.6$^{\circ}$N latitude and 81.63$^{\circ}$E longitude at an altitude of 298 meters above the sea level under Chhattisgarh plains.

**3.2 Climatic condition:**

The climate of this region is sub-humid to semi-arid. The average annual rainfall ranges from 1200- 1400 mm, out of which 85 per cent rainfall is received during middle of June and by the end of September and very little during October to May. The maximum temperature goes as high as 48$^{\circ}$C during the month of May and minimum as low as 6$^{\circ}$C in winter months of December-January.

The details regarding materials used and techniques applied during the course of investigation are described objective wise briefly as under:-

## 3.3 To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.

### 3.3.1. Rearing of Greater wax moth, *Galleria mellonella* as host for isolation and mass multiplication of EPNs

**Common name:** Greater wax moth

**Scientific name:** *Galleria mellonella* (Linnaeus, 1758)

**Order:** Lepidoptera

**Family:** Pyralidae

EPNs have been successfully cultured on greater wax moth, *G. mellonella* reared under laboratory conditions on standard artificial diet. Collected adult moths were placed in plastic jars and fed with honey solution. Adult female wax moths mated with males were allowed to lay eggs on a lining of tissue paper. Later, provided with separate plastic jars for hatching of *G. mellonella* larvae. The hatched larvae were maintained on artificial diet composed of following ingredients -

| | | |
|---|---|---|
| Maize flour | - | 250 gm |
| Wheat flour | - | 250 gm |
| Wheat/rice bran | - | 250 gm |
| Milk powder | - | 120 gm |
| Yeast powder | - | 15 gm |
| Honey | - | 200 ml |
| Glycerol | - | 200 ml |

#### 3.3.1.1. Materials required for rearing of Greater wax moth

| Items | Care to be taken | Picture |
|---|---|---|
| **Weighing balance** | Avoid approximate usage of all the ingredients |  1 |

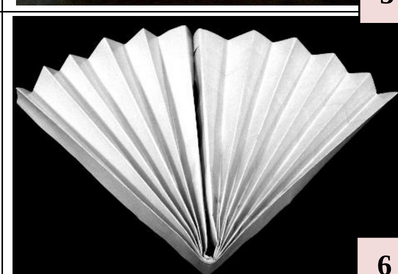
| | | |
|---|---|---|
| **Plastic tub** | Size can be chosen based on diet quantity required |  2 |
| **Plastic beaker** | For mixing thoroughly honey and glycerol |  3 |
| **Plastic jar for wax moth breeding.** | Plastic jar lid to be cut with a hot knife in a circular fashion and joined with a fine metal mesh with the help of a hot edge of a knife to provide proper ventilation to the moths. |  4 |
| **Plastic jar for rearing larvae** | Plastic jar lid to be cut with a hot knife in a circular fashion and joined with a fine metal mesh with the help of a hot edge of a knife to provide proper ventilation to the larvae and diet should not exceed 6cm height. |  5 |
| **Collection of eggs through lining tissue papers** | After noticing moth emergence from the pupa, allow moth to lay eggs on lining of tissue papers dropped into the plastic jars to allow the moths lay eggs on the paper sheets. |  6 |

**Plate No. 1. *G. mellonella* rearing materials (1. Weighing balance, 2. Plastic tub, 3. Plastic beaker, 4. Plastic jar for breeding., 5. Plastic jar for rearing larvae 6.lining of tissue papers.)**

**3.3.1.2. Protocol for the mass multiplication of Greater wax moth**

Select healthy late instars *G. mellonella* larvae for pupation into a separate plastic jars preferably with artificial diet for adult emergence

⇩

Place the folded lining of tissue paper in plastic jars for egg laying.

⇩

Collect the egg masses carefully by replacing the lining tissue paper once in two days for six days with fresh paper.

⇩

Cut the lining of tissue paper adhered with egg masses then transfer the pieces of paper in to a plastic jars along with pieces of honey comb or diet to facilitate the egg hatching.

⇩

Incubate the plastic jars at 28°C for a week period of time for egg hatching.

⇩

After 10-12 days of incubation, transfer the young larvae to plastic jars with the artificial diet.

⇩

Incubate the plastic jars for 12-15 days for the larvae to gain body weight and add diet at intervals (during this period fresh diet should be provided for fast and health growth of larvae).

⇩

Harvest the larvae once it attains the body weight 300 mg or more for mass production of EPNs.


**Plate No. 2 Life cycle of *Galleria mellonella***

### 3.3.2 Collection of soil samples

Survey for isolation of entomopathogenic nematodes was carried out during June to September, 2017 - 18. Soil samples were collected from different districts under different agro-ecosystems belonging to the three agro-climatic zones of Chhattisgarh, Out of which, 15 samples were from Northern hills, 56 samples from Chhattisgarh plains and 11 samples from Bastar plateau belonging to different cropping systems like rice, sorghum, maize, bajra, cotton; vegetable crops like tomato, brinjal, lady-finger; ornamental crops like jasmine and rose; fruit crops like mango, citrus and guava etc, during the course of study.

Soil sampling for EPN isolation was done by using random sampling. Tools like spade and hand shovel were used for collecting soil samples at 15 cm depth around the root zone of the crops by V-notch method. From the composite sample, 500 gm of soil was finally drawn for baiting placed in polyethylene bag with few pin-head size perforations for aeration and labelled with a waterproof permanent marker including site information (crop name, locality and date,) etc.

### 3.3.3 Isolation of EPNs through baiting technique

The soil samples were brought to the Biocontrol laboratory and transferred into small plastic containers and each container was baited with two to three final instar larvae of *Galleria mellonella*. Sample containers were covered tightly with lids containing small holes to facilitate gaseous exchange and were kept at room temperature (25 ± 2°C). After two days, if the larvae got infested with EPNs, the cadaver colour changed to brick red or grey. Then, it was separated and placed in small petriplates on moist filter paper (Whatman No.1) as White trap for emergence of EPNs from the cadavers. The emerging nematodes *i.e.* infective juvenile stages (IJs) were re-inoculated into *G. mellonella* larvae, to get pure IJs.

Soil sample collection for EPN's isolation from different districts under different agro-ecosystem belonging to the three agro-climatic zones of Chhattisgarh are illustrated in Plate No.3 and Plate No.4.

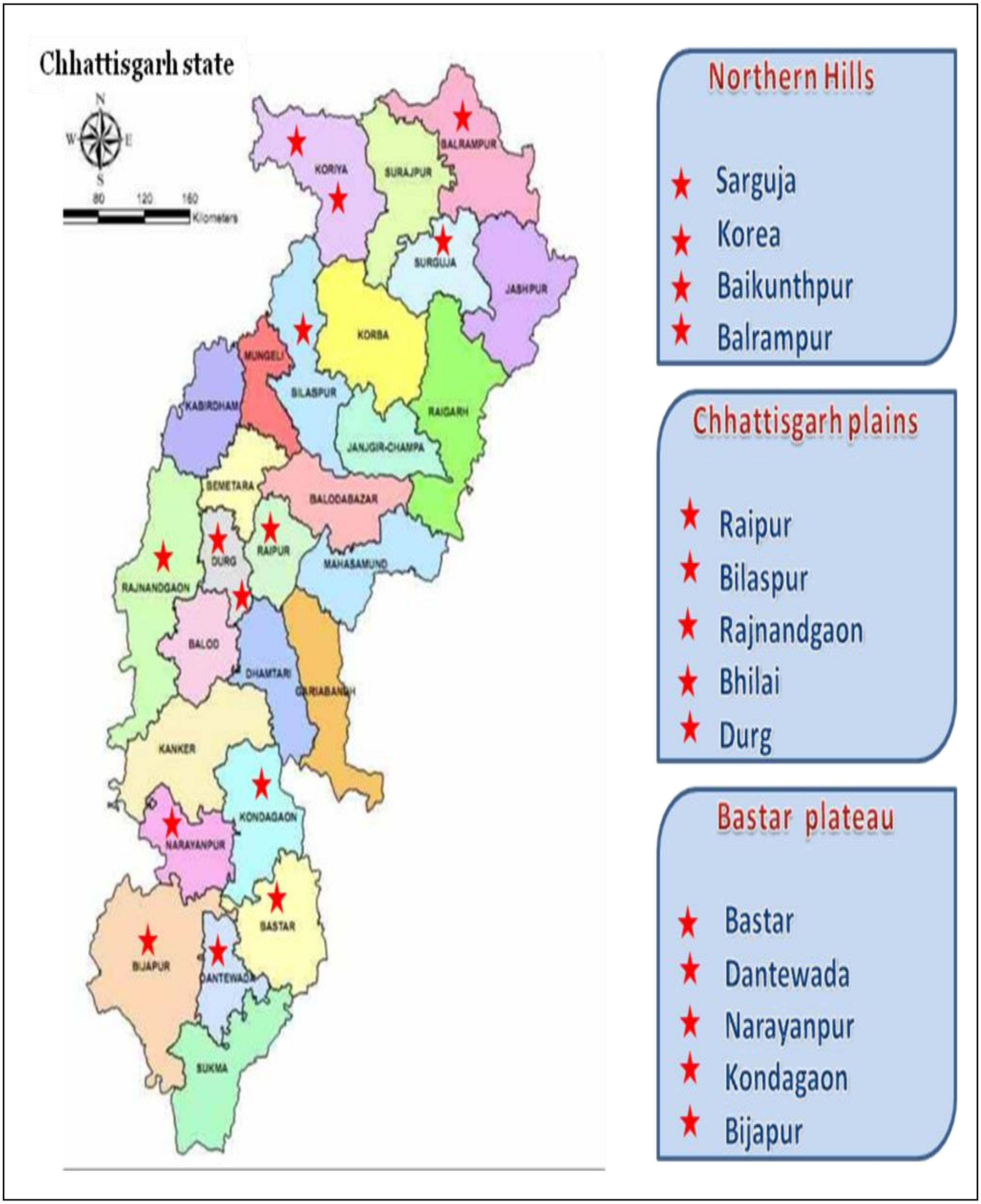



**Plate No. 3. Soil samples collection for EPNs isolation at different districts of Chhattisgarh.**

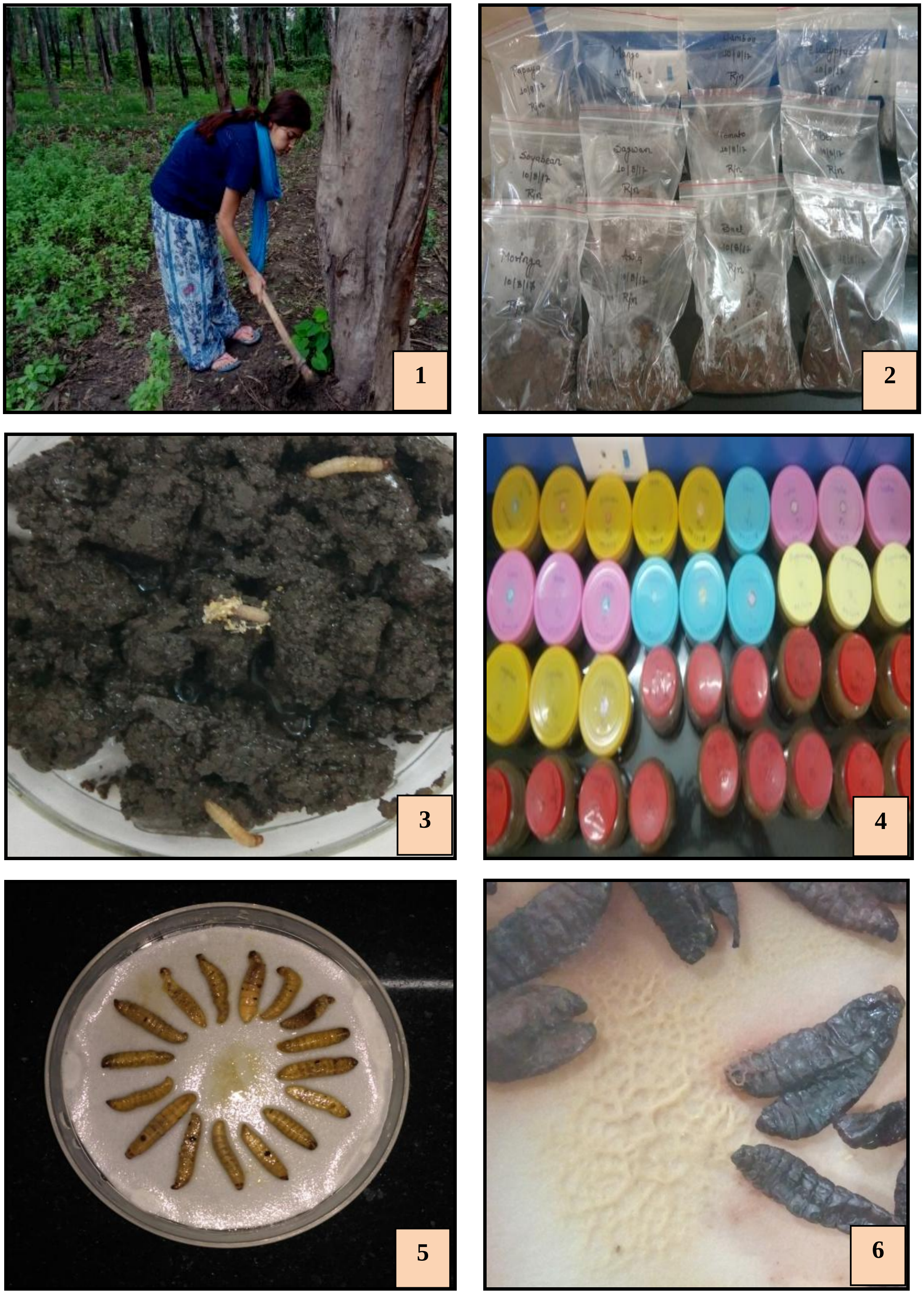



**Plate No. 4. Collection of soil samples and isolation of EPNs. (1. Soil sampling 2. Collected sample 3. Baiting techniques 4. Placed in small vials 5. White trap method 6. Infected cadaver with IJs emergence)**

### 3.3.4. Preparation of white trap

Watch glass (52 mm diameter) was placed on large petri dish (110×25 mm) in inverted position and then a circular filter paper (Whatman no.1) was placed on the watch glass in such a way that, the lower edge of the filter paper touched the bottom of the petri dish. Then, distilled water was poured over the filter paper and the level of the water was maintained always to be in contact with the edges of the filter paper. After that infected cadavers were placed on the top of the watch glass and it was covered finally on top by the lid of the petri dish.

### 3.3.5. Harvesting of EPNs

Four days after incubation the infected larvae were placed on white trap. The infected cadavers were placed on the watch glass and the petridish was covered with parafilm and kept in incubator at room temperature 25 ± 2°C. Since the first emergence, IJs were harvested with distilled water in to a beaker upto four to five days. Harvested IJs were washed with 0.1 per cent hymine solution, filtering through filter paper. In this manner it was cleaned three to four times with distilled water. From the total IJs solution 50µl was pippetted with micropipette and counted numbers of IJs were harvested. Freshly harvested IJs were stored in a tissue culture flask mixing with 2 per cent formalin to increase stability.

### 3.3.6. Identification

Identification of the EPNs infection was done based on the colour change of the test insect. In case of *Steinernema carpocapsae,* the infected cadavers turned to grey and *Heterorhabditis indica* infected cadavers turned to brick red. The EPNs emerging from soils of different agro-ecosystems and locations were transferred to petridish containing autoclaved sterilized soil and sand in the ratio of 1:1 and provided with two *G. melonella* larvae/ sample were sent for identification to NBAIR, Bengaluru.

The EPNs were identified through either morphological or molecular character basis. The slide preparation of EPNs for studying their key characters, the following chemical solutions were used.

**Reagent:-**

1. **Ringer's solution (pH - 7.3)**

   Sodium chloride (NaCl) – 9 g

   Potassium chloride (KCl) – 0.4 g

   Calciumchloride (CaCl) – 0.4 g

   Sodium hydrogen carbonate ($NaH_2CO_3$) – 0.2 g

   Distilled water -1000 ml

2. **Killing and fixing fixative (Formalin glycerol fixative):**

   Formalin (40% formaldehyde) – 8 ml

   Anhydrous glycerol – 2 ml

   Distilled water -1000 ml

3. **Killing and fixing fixative ( TAF):**

   Formalin (40% formaldehyde) – 7 ml

   Triethanolamine – 2 ml

   Distilled water - 91 ml

4. **Seinhorst solution (dehydrating agent)**

   Ethanol 95% - 20 ml

   Anhydrous glycerol – 1 ml

   Distilled water -79 ml

**Killing and fixing:**

The methods for killing, fixing and slide preparation of EPNs for studying their key characters, involved the following procedure:

- Cadavers were dissected in Ringer's solution and EPNs were collected in a 4 ml glass vial filled with ringer's solution with screw cap which removes insect debris.
- Vials were placed in a water bath at $65^{o}C$ for 2 min.

- The fixative (TAF) is added to an equal amount of Ringer's solution (1:1) when the nematodes were killed.
- Killed nematodes were placed in small container with TAF and Ringer's solution (1:1) in the water bath at 65$^{o}$C.

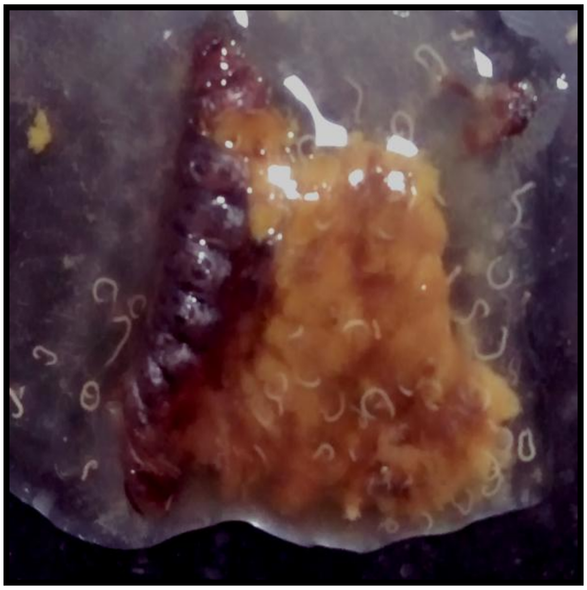
**Slide preparation:**

- Cadavers were dissected in Ringer's solution or 1% saline solution.
- Adult nematodes collected from the cadavers were put into cavity blocks with fixative and kept for 24 hours.
- Fixative was removed with the help of a syringe from cavity block.
- Seinhorst solution was added and kept for 3-4 weeks in dessicator.
- Cavity block was removed from dessicator.
- Single nematode was picked and placed on the slide.
- Pin drop size anhydrous glycerine was added and covered with cover slip on slide warming table (60$^{o}$C – 75$^{o}$C) and melted wax was used to seal the slide.

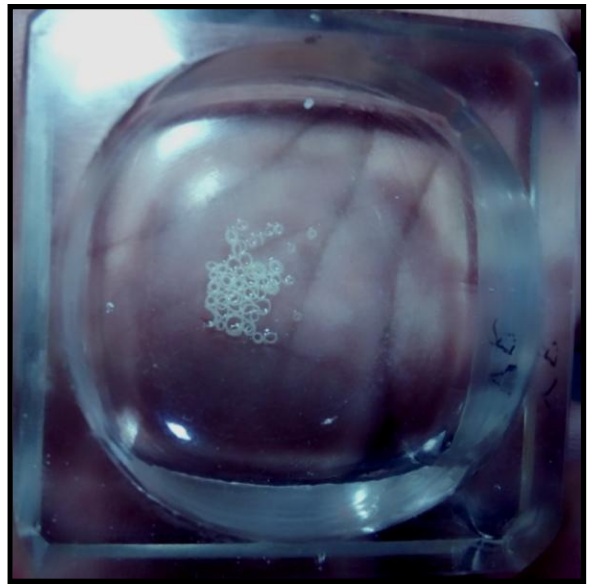
***S. carpocapsae* infected cadavers**

***H. indica* infected cadavers**

**Plate No. 5. EPNs infected cadavers**

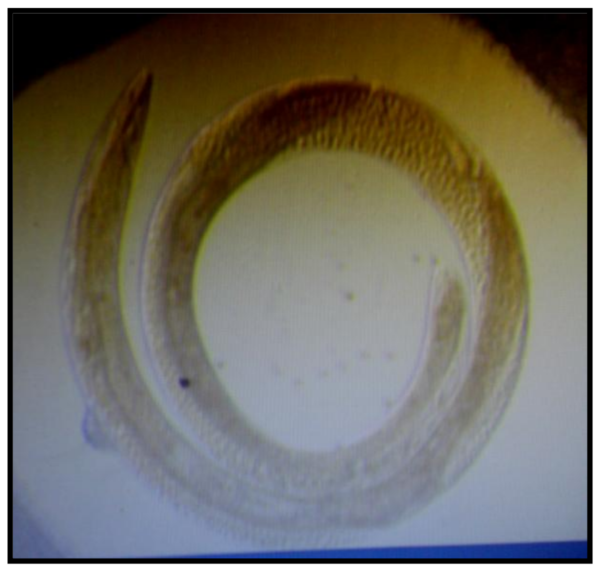


Killing and dissecting of cadaver



Dessicated EPNs on cavity block

Slide preparation



Adult EPNs

**Plate No. 6. Killing, fixing and slide preparation being done at NBAIR, Bengaluru.**

### 3.4 To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh.

Experiments were carried out under laboratory conditions by using the two genera of entomopathogenic nematodes *i.e. S. carpocapsae* and *H. indica*. Observations were recorded on the effect of the factors such as variable temperature, ultra-violet (UV) rays, pH, soil depth and soil texture etc on the survival of EPNs. For initiating the experiment, the number of EPNs present in the stock solution was calculated by using the formula as per (Anonymous, 2013).

#### 3.4.1. To calculate the actual number of nematodes in the stock solution was calculated by using the formula (Anonymous, 2013).

$$C = N \times (X+1) \times S$$

Where,

C = Actual number of nematodes in the stock solution.

N = Average number of nematodes per counted sample.

S = Volume of original stock solution (in ml).

(X+1)= Dilution.

#### 3.4.2. To prepare dilution with a given number of nematodes per ml, from the counted suspension, the following formula was used (Anonymous, 2013)

$$A = D \times C/B$$

Where,

A = ml of suspension of known concentration, the suspension to be diluted.

B = Number of nematodes per ml in suspension to be diluted.

C = Final volume in ml of new dilution.

D = Desired concentration in new dilution.

#### 3.4.3. Percent mortality of nematodes in a known stock solution was calculated by using the following formula (Anonymous, 2013)

$$M = \frac{Y \times (X+1) \times S}{T} \times 100$$

Where,

M = Percent mortality.

Y = Average number of dead nematodes per counted sample.

S = Volume of original stock solution (in ml).

T = Total number of nematodes in the original stock solution

(X+1)= Dilution.

**3.4.4. Effect of the following parameters was studied:-**

**i) Temperature**

The experiment for testing the survival of EPNs at different range of temperature *i.e.* 5$^o$C, 10$^o$C, 15$^o$C, 20$^o$C, 25$^o$C, 30$^o$C and 35$^o$C at duration of 24 to 96 hours in BOD incubator replicated four times.

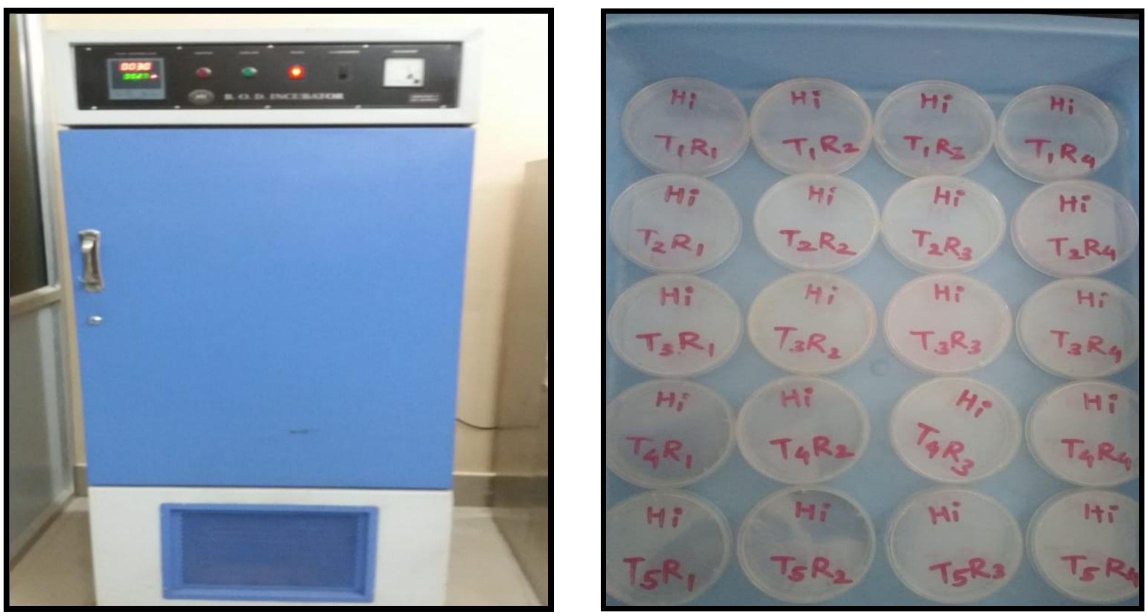


**Plate No. 7. Experimental setup for studying the temperature effect on EPNs survival.**

Counted numbers of EPNs in 0.5 ml with 1.5 ml distilled water and made up to 2 ml solution, were taken in petridishes and exposed to different range of temperature. Observations were recorded from one ml of each replicate after 24 hours of every treatment through shaking of EPNs solutions and then observed under microscope for recording survival and mortality of the IJs.

**ii) Ultraviolet radiation**

Testing the survival and mortality of EPNs towards Ultraviolet radiation by exposing the counted numbers of EPNs / µl to UV radiation (at an average of 254 nm wave length) for different duration of time *i.e.* 15, 30, 45, 60, 75, 90, 105 and 120 minutes in four replications along with control in a laminar air flow.

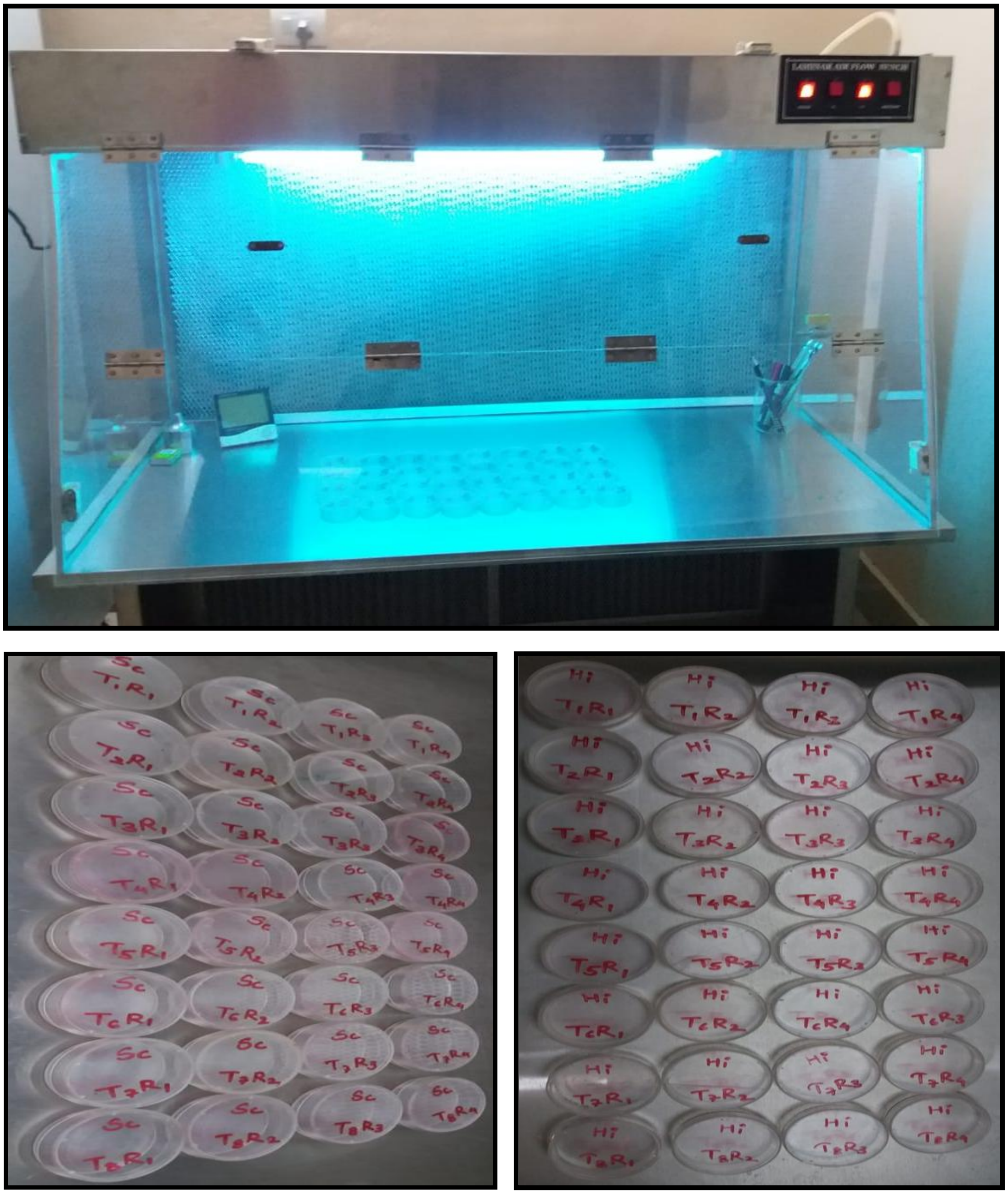


**Plate No. 8. Experimental setup for studying the U.V. radiation effect on EPNs survival**

The experiment on the effect of UV radiation was carried out under laboratory conditions, using sterilized petriplates into which 1000 IJs in 0.5 ml of IJs released into 1.5 ml distilled water and made into 2 ml solution and placed in a laminar air flow chamber under UV light. Petriplates with IJs were kept outside the chamber at room temperature maintained as control.

**iii) pH**

The survival and mortality of EPNs at different ranges of pH were done by taking counted number of EPN's/ µl and by exposing to different levels of pH ranging from 4.00 to 9.00 replicated four times along with control tested in the laboratory conditions.

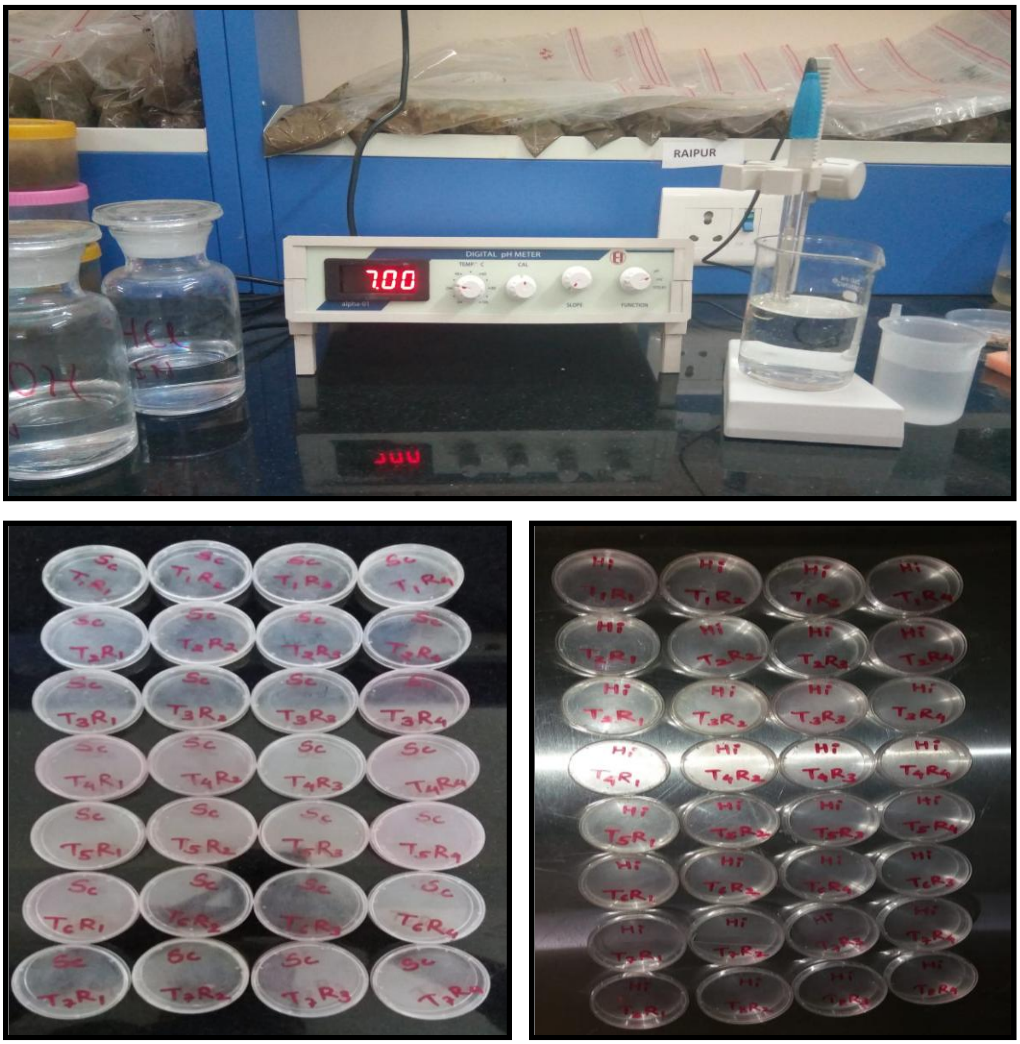



**Plate No. 9. Experimental setup for studying the effect of pH on survival of EPNs.**

The pH range from 2.00 to 9.00 was adjusted with sodium hydroxide for alkality and hydrochloric acid for acidity required. The pH of samples was tested with table type pH meter. Tests were conducted on petriplates and each petriplate was filled with 2 ml suspension containing 1000 IJs. The survival of IJs was recorded till 28 days at weekly intervals.

**vi) Soil depth**

For studying the movement of EPNs for host searching at different depth of soil were tested by using PVC pipes of different lengths such as 5.00, 10.00, 15.00, 20.00, 25.00, 30.00 and 35.00 cms to observe the movement of EPNs at different soil depth and soil type *i.e.* sandy soil, sandy loam and clay soil replicated thrice**.**

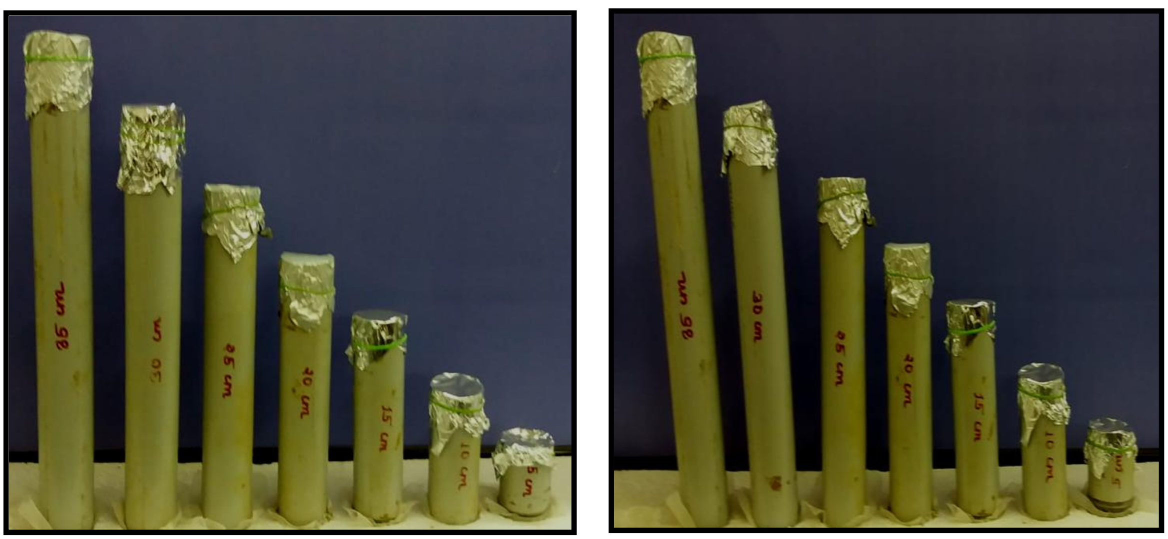


**Plate No.10 Experimental setup for studying the vertical movement of EPNs at different depth on various type of soil.**

### 3.5 To study the mass multiplication of EPNs at laboratory condition on different hosts.

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*.

### 3.5.1. Mass multiplication of EPNs on *G. mellonella* by inoculating with different doses of IJs.

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, 80 and 100 IJs 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 ± 2°C for release of nematodes.

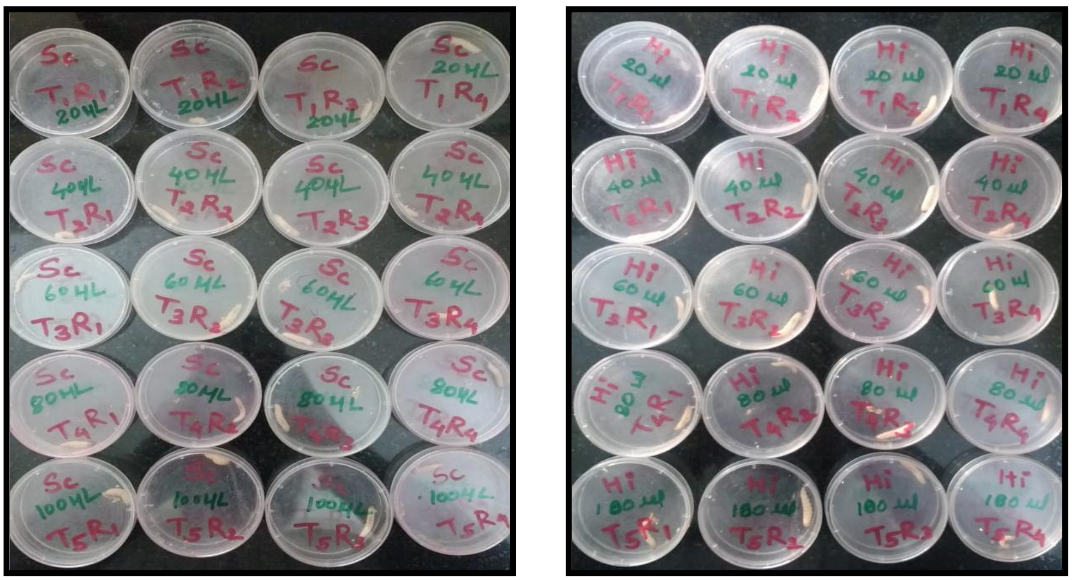
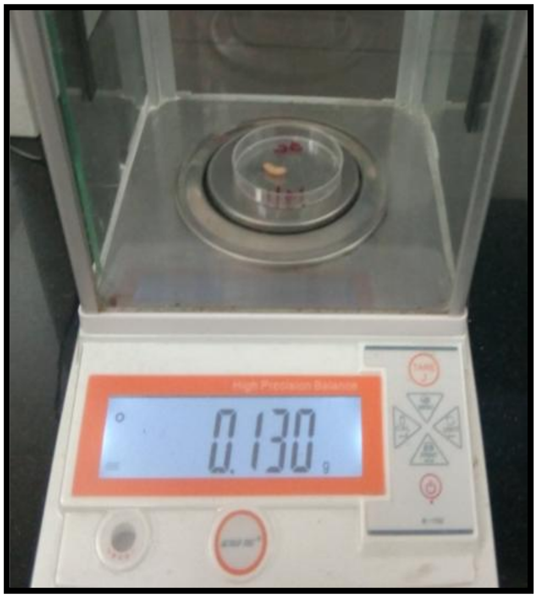


**Plate No. 11. Experimental setup for studying the mass multiplication of EPNs on *G.mellonella* by inoculating with different doses of IJs.**

### 3.5.2. Progeny production of EPNs from different sized larvae of *G. mellonella.*

For finding suitable size of the final instar larvae of *G. mellonella,* larvae of different sizes were used, based on the length and weight. For this experiment, ten small sized (body length of 9-11mm and weight of 140-145 mg), medium sized (body length of 15-16 mm and weight of 210-220 mg) and large sized (body length of 20-21 mm and weight of 300-315 mg) larvae were taken into separate petriplates over a filter paper.

Then larvae were inoculated separately with infective juveniles of *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 ± 2°C and the IJs emerged were harvested.



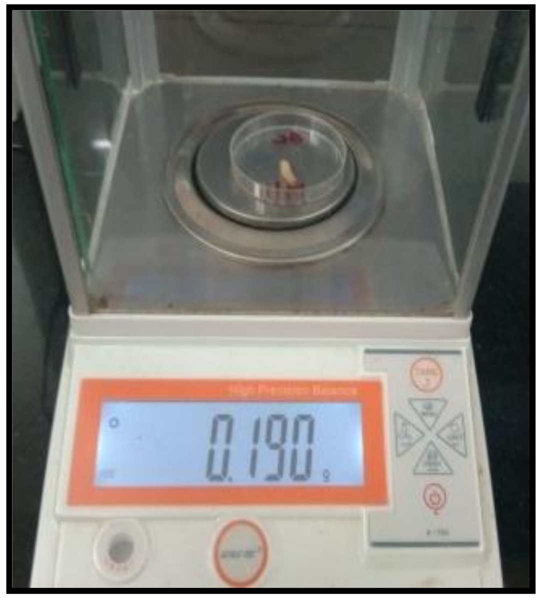




**Plate No. 12. Experimental setup for studying the progeny production of EPNs from different sized larvae of *G. mellonella.***

### 3.5.3. Progeny production of EPNs from different insect hosts

EPNs trapped through insect baiting techniques were mass multiplied on different host insect pests. Experiments were conducted to find out the most suitable host insect for mass multiplication of *S. carpocapsae* and *H. indica* under laboratory conditions. For this experiment, four larvae each of the final instar of *G. mellonella*, *Corcyra cephalonica, Helicoverpa armiger*a and *Spodoptera litura* were taken into petridish over moist filter paper and inoculated with 1 ml suspension (60 IJs / larvae) of *S. carpocapsae* and *H.indica* separately, replicated four times. Then, the dead cadaver were transferred to white trap and kept for incubation. Emerged juveniles were harvested.

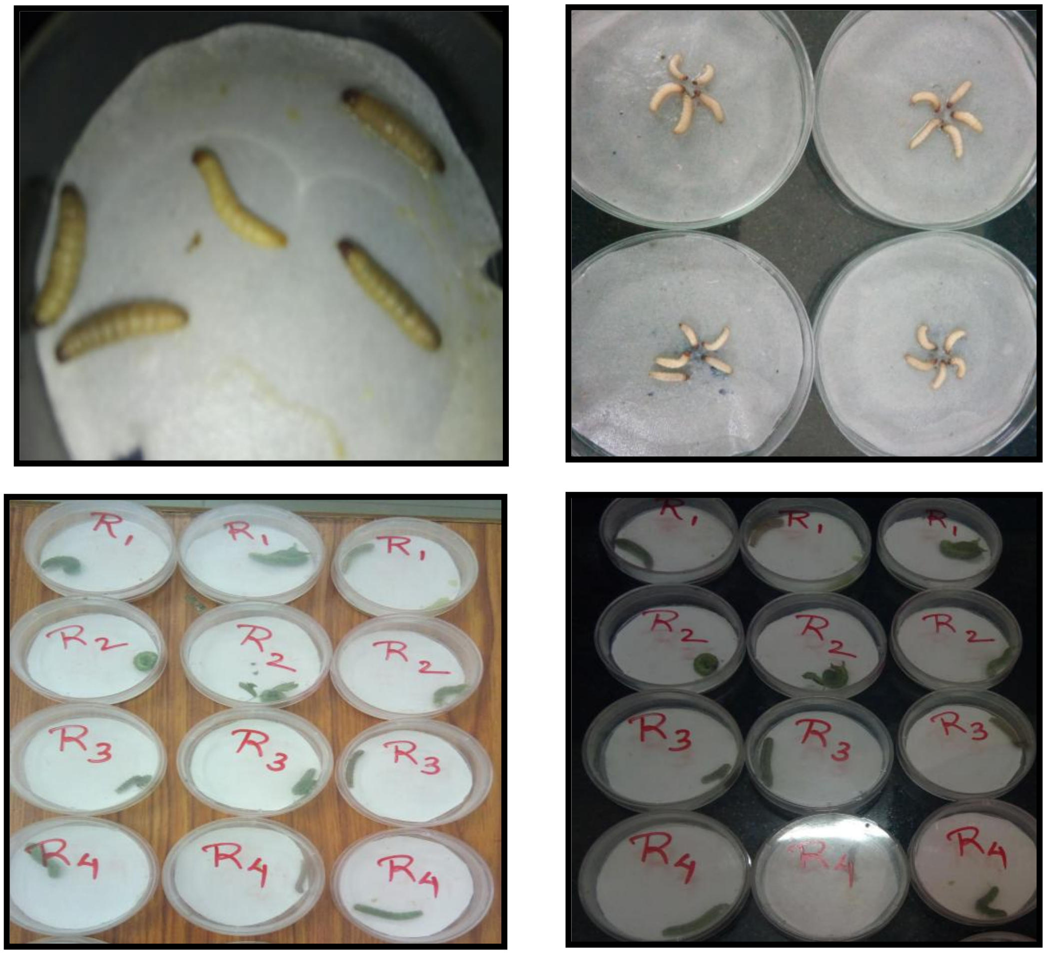


**Plate No.13. Experimental setup for studying the progeny production of EPNs from different insect hosts (1. *G. mellonella* 2. *Corcyra cephalonica* 3. *Helicoverpa armiger*a 4. *Spodoptera litura*)**

### 3.5.4. Harvesting of nematodes

Four days after incubation the infected larvae were placed on white trap. The infected cadavers were placed on the watch glass and the petridish was covered with parafilm and kept in incubator at room temperature 25 ± 2°C. Since the first emergence, IJs were harvested with distilled water in to a beaker upto four to five days. Harvested IJs were washed with 0.1 per cent hymine solution, filtering through filter paper. In this manner it was cleaned three to four times with distilled water. From the total IJs solution 50µl was pipetted with micropipette and counted for number of IJs were harvested.

# CHAPTER – IV
# RESULT AND DISCUSSION

This chapter deals with the results and discussion of the work done under different objectives of the experiment entitled, **"Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh"** conducted in the Biological control laboratory of Department of Entomology, College of Agriculture - Raipur, Indira Gandhi Krishi Vishwavidyalaya Raipur (C.G.) during the year 2017-18. The results are presented under the following sub headings:

**Objectives:-**

1. To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.
2. To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh.
3. To study the mass multiplication of EPNs at laboratory condition on different hosts.

## 4.1 To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.

Soil samples were collected for isolation and identification of EPNs from different districts under different agro-ecosystems belonging to the three agro-climatic zones of Chhattisgarh illustrated in Table No-4.1 and Plate No.-14.

### 4.1.1 Isolation of EPNs

Soil samples were collected for isolation of EPNs from different districts under different agro-ecosystems belonging to the three agro-climatic zones of Chhattisgarh Table No-4.1 and Plate No.-14. EPNs isolation survey was carried out during June to September, 2017 – 18 through soil baiting technique by *G. mellonella* larvae. Total 82 soil samples were collected from all the three agro-climatic zones of Chhattisgarh. Out of 82 soil samples, 13 from Northern hills, 56 from Chhattisgarh plains and 13 soil samples were collected from Bastar Plateau of different cropping systems of Chhattisgarh. EPNs could be recovered from only two soil samples of Chhattisgarh plains by body discolouration and mortality of *G. mellonella* larvae within 24 - 48 hours under laboratory conditions through soil baiting technique indicated the presence of EPNs from those respective samples.

In Chhattisgarh plains, out of 56 soil samples only two soil samples were having positive response towards body discolouration of larvae and caused mortality within 48 hours. Body colour of larvae changed from greyish to black which indicated the presence of IJs of *Steinernema carpocapsae* collected from the soil samples of yam ecosystem at Raipur district and brick red colour indicated the presence of IJs of *Heterorhabditis indica* collected from the soil samples of rose ecosystem from Durg district. After a week IJs emerged from the cadaver.

### 4.1.2 Identification of EPNs

EPNs were identified from the soil samples that have been collected in and around the different districts under different agro-ecosystems belonging to the three agro-climatic zones of Chhattisgarh. For confirmation of the presence of EPNs, the collected samples were sent to National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru at Karnataka. Results confirmed from the sent soil samples, two isolates of EPNs as *S. carpocapsae* and *H. indica.* Both the isolates were isolated from the loamy soil. *S. carpocapsae* isolates were identified from the soil samples of yam ecosystem at Raipur district and *H. indica* isolates were identified from the rose ecosystem of Durg district in Chhattisgarh.

The present study recorded for the occurrence of EPNs in Chhattisgarh. Although, EPNs were recovered from only 2 out of 82 samples *i.e.* 2.43 per cent the recovery highlights the importance of conducting more intensive survey for isolation of EPNs. The recovery of EPNs from 2 locations in Chhattisgarh is an indication of the suitable climate for the multiplication and survival of EPNs. The climate of Chhattisgarh must have supported the survival and prevalence of host insects and thus ultimately satisfactory occurrence of EPNs especially in perennial crops due to shade and higher moisture under the canopy of the trees.

**Table No. 4.1 Isolated and identified species of EPNs**

| S.No. | Districts | Crop | Soil type | Identified species |
|---|---|---|---|---|
| | | **Northern hills** | | |
| **1.** | **Sarguja** | Guava | Loamy soil | -- |
| | | Pear | Clay soil | -- |
| | | Papaya | Clay soil | -- |
| | | Mango | Clay soil | -- |
| | | Litchi | Red soil | -- |
| | | Peach | Clay soil | -- |
| | | Maize | Loamy soil | |
| **2.** | **Korea** | Mango | Red soil | -- |
| | | Guava | Loamy soil | -- |
| **3.** | **Baikunthpur** | Rice | Clay soil | -- |
| | | Pigeon pea | Loamy soil | |
| **4.** | **Balrampur** | Rice | Clay soil | -- |
| | | Ber | Loamy soil | -- |
| | | **Chhattisgarh plains** | | |
| **5.** | **Raipur** | Sagwan | Clay soil | -- |
| | | Anola | Clay soil | -- |
| | | Aloevera | Clay soil | -- |
| | | Eucalyptus | Clay soil | -- |
| | | Okra | Loamy soil | -- |
| | | Maize | Loamy soil | -- |
| | | Sugarcane | Clay soil | -- |
| | | Cucurbit | Clay soil | -- |
| | | Yam | Loamy soil | ***Steinernema carpocapsae*** |
| | | Marigold | Loamy soil | -- |
| | | Organic rice | Clay soil | -- |
| | | Tulsi | Clay soil | -- |

| | | | | |
|---|---|---|---|---|
| | | Groundnut | Clay soil | -- |
| | | Arhar | Loamy soil | -- |
| | | Soyabean | Clay soil | -- |
| | | Tomato | Loamy soil | -- |
| **6.** | **Bilaspur** | Cucurbit | Loamy soil | -- |
| | | Citrus | Clay soil | -- |
| | | Guava | Clay soil | -- |
| | | Moringa | Loamy soil | -- |
| | | Sal | Clay soil | -- |
| | | Karonda | Clay soil | -- |
| | | Ber | Clay soil | -- |
| | | Custard apple | Clay soil | -- |
| | | Mango | Clay soil | -- |
| | | Rose | Loamy soil | -- |
| | | Davana | Clay soil | -- |
| | | Bamboo | Clay soil | -- |
| **7.** | **Rajnandgaon** | Sagwan | Loamy soil | -- |
| | | Bael | Clay soil | -- |
| | | Anola | Loamy soil | -- |
| | | Jamun | Loamy soil | -- |
| | | Mahua | Clay soil | -- |
| | | Khamar | Clay soil | -- |
| | | Ber | Clay soil | -- |
| | | Eucalyptus | Clay soil | -- |
| | | Bamboo | Loamy soil | -- |
| | | Mango | Clay soil | -- |
| | | Papaya | Clay soil | -- |
| | | Moringa | Loamy soil | -- |
| | | Soyabean | Clay soil | -- |
| | | Tomato | Loamy soil | -- |
| **8.** | **Bhilai** | Aloevera | Clay soil | -- |

| | | | | |
|---|---|---|---|---|
| | | Banana | Clay soil | --- |
| | | Ber | Clay soil | -- |
| | | Bamboo | Clay soil | -- |
| | | Neem | Clay soil | -- |
| | | Pomegranate | Loamy soil | -- |
| | | Guava | Clay soil | -- |
| | | Tulsi | Clay soil | -- |
| **9.** | **Durg** | Papaya | Clay soil | -- |
| | | Mango | Clay soil | -- |
| | | Guava | Clay soil | -- |
| | | Aloevera | Loamy soil | -- |
| | | Rose | Loamy soil | ***Heterorhabditis indica*** |
| | | Mung | Loamy soil | -- |
| | | **Bastar Plateau** | | |
| **10.** | **Bastar** | Eucalyptus | Clay soil | -- |
| | | Sal | Clay soil | -- |
| | | Teak | Loamy soil | -- |
| | | Ber | Loamy soil | -- |
| **11.** | **Dantewada** | Rice | Clay soil | -- |
| | | Groundnut | Clay soil | -- |
| **12.** | **Narayanpur** | Jamun | Loamy soil | -- |
| | | Bamboo | Clay soil | -- |
| **13.** | **Kondagaon** | Maize | Loamy soil | -- |
| | | Rice | Clay soil | -- |
| | | Pigeon pea | Clay soil | -- |
| **14.** | **Bijapur** | Groundnut | Sandy loam soil | -- |
| | | Rice | Clay soil | -- |

**Isolated and identified species of EPNs**

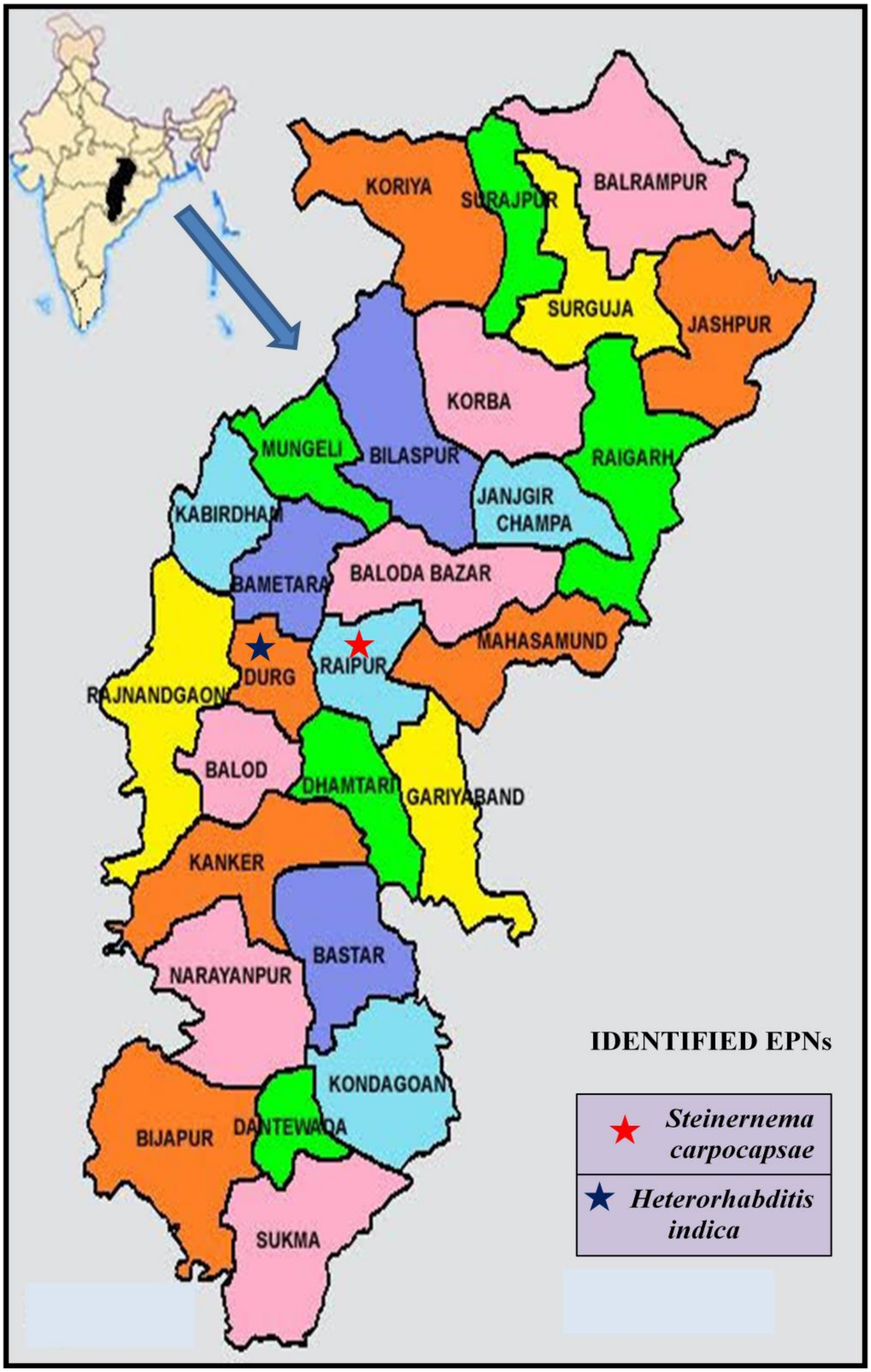



**Plate No.14 Isolated and identified species of EPNs**

This is similar to the findings of Rajkumar *et al.* (2001) who also reported presence of EPNs from five samples from 105 soil samples collected from Udaipur district of Rajasthan *i.e.* 4.76 per cent. Similar results were also reported by Parihar *et al.* (2002) mentioning 0.62 per cent *Steinernema* sp. and 0.83 per cent Heterorhabditid from Rajasthan. Hazir *et al*. (2003) also reported a single EPN species from each of the 2.03 per cent positive sites. Present results match with the findings of Dubey *et al.* (2010) who reported that out of 29 soil samples, one sample isolate from rhizosphere of sapota in Agriculture Research Farm, Indira Gandhi Krishi Vishwavidyalaya, Koni, Bilaspur, revealed the presence of EPNs *i.e.* IARI-EPN-*chh*1 which was most closely related to *S. carpocapsae* or might be representing a new species of *Steinernema* in Chhattisgarh.


**Plate No. 15. EPNs infected cadavers and infective juveniles ($J_3$) (1. *S. carpocapsae* infected cadavers 2. *H. indica* infected cadavers 3. Infective juveniles ($J_3$) of *S. carpocapsae* 4. Infective juveniles ($J_3$) of *H. indica*).**

## 4.2 To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh

### 4. 2.1 Effect of temperature on EPNs:-

#### 4.2.1.1 Effect of temperature on survival and mortality of EPNs

Experiments conducted for observing the influence of different levels of temperature *i.e.* 5, 10, 15, 20, 25, 30 and 35°C on survival and mortality of the IJs of the EPNs of the two species *i.e. S. carpocapsae* and *H. indica*, revealed that with increasing level of temperature there was gradual decrease in survival per cent.

#### 4.2.1.1.1 Effect of temperature on survival and mortality of *S. carpocapsae*

Results of the average effect of temperature on survival of the EPNs under laboratory conditions revealed that temperature of 25°C was the optimum temperature that showed maximum 99.60 per cent survival of the IJs of *S. carpocapsae*. With increase in temperature to 30°C and 35°C the percentage of survival 82.93 and 44.93 per cent respectively was observed. When temperature was decreased from 20°C, 15°C, 10°C, 5°C survival rates of IJs ranged from and 98.88, 98.36, 97.17 and 95.40 per cent respectively.

Data presented on survival of *S. carpocapsae* in the Table No. 4.2 showed that, the maximum mean survival 99.60 per cent of IJs was observed at 25°C from 24 to 96 hours of exposure. With increase in temperature to 30°C and 35°C the mean survival per cent of IJs reduced further significantly from 82.93 and 44.93 per cent.

As far as the percentage reduction in survival of the IJs was concerned, the data in Table No. 4.2 indicates, lesser decrease in survival with the decrease in temperature from 25°C, 20°C, 15°C, 10°C and 5°C as 0.40, 1.12, 1.64, 2.83 and 4.60 per cent as compared to increase in temperature from 25°C to 30°C and 35°C depicted a greater decrease of 0.40 to 17.07 and 55.07 per cent respectively.

Thus, from the above data, it can be concluded that *S. carpocapsae* showed less survival with increase in temperature as compared to decrease in temperature, which also justifies its presence in lesser number in hotter climatic conditions as of Chhattisgarh.

**Table No. 4.2 Effect of temperature on mortality and survival percentage of *S. carpocapsae***

| Temp. | 24 hrs. | | 48 hrs. | | 72 hrs. | | 96 hrs. | | Mean | | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| °C | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Reduction in survival |
| **5 °C** | 2.75 | 97.25 | 3.25 | 96.75 | 5.12 | 94.87 | 7.25 | 92.75 | 4.59 | 95.40 | 4.60 |
| | (9.49) | (80.47) | (10.34) | (79.62) | (13.07) | (76.88) | (15.60) | (74.36) | | | |
| **10 °C** | 1.55 | 98.45 | 1.95 | 98.05 | 3.25 | 96.75 | 4.55 | 95.45 | 2.82 | 97.17 | 2.83 |
| | (7.09) | (82.86) | (8.01) | (81.95) | (10.34) | (79.62) | (12.30) | (77.66) | | | |
| **15 °C** | 0.97 | 99.03 | 1.15 | 98.75 | 1.70 | 98.30 | 2.52 | 97.47 | 1.58 | 98.36 | 1.64 |
| | (5.64) | (84.31) | (6.14) | (83.35) | (7.48) | (82.48) | (9.12) | (81.02) | | | |
| **20 °C** | 0.61 | 99.38 | 0.77 | 99.22 | 1.35 | 98.65 | 1.72 | 98.27 | 1.11 | 98.88 | 1.12 |
| | (4.47) | (85.48) | (5.04) | (84.92) | (6.61) | (83.35) | (7.53) | (82.42) | | | |
| **25 °C** | 0.00 | 100.00 | 0.00 | 100.00 | 0.60 | 99.40 | 0.97 | 99.03 | 0.39 | 99.60 | 0.40 |
| | (0.12) | (90.00) | (0.12) | (90.00) | (4.41) | (85.54) | (5.64) | (84.31) | | | |
| **30 °C** | 9.25 | 90.75 | 15.25 | 84.75 | 19.87 | 80.12 | 23.87 | 76.12 | 17.06 | 82.93 | 17.07 |
| | (17.69) | (72.27) | (22.97) | (66.99) | (26.46) | (63.50) | (29.23) | (60.72) | | | |
| **35 °C** | 40.75 | 59.25 | 47.75 | 52.25 | 60.60 | 40.25 | 72.25 | 27.98 | 55.33 | 44.93 | 55.07 |
| | (39.65) | (50.31) | (43.69) | (46.27) | (59.75) | (39.36) | (62.18) | (21.75) | | | |
| **Control** | 0.00 | 100.00 | 0.00 | 100.00 | 0.00 | 100.00 | (0.00) | 100.00 | 0.00 | 100.00 | - |
| **(25±2°C)** | (1.00) | (90.00) | (1.00) | (90.00) | (1.00) | (90.00) | (1.00) | (90.00) | | | |
| **SE(m)** | 0.31 | 0.31 | 0.26 | 0.32 | 0.33 | 0.33 | 0.25 | 0.45 | - | - | - |
| **C.D.(1%)** | 0.92 | 0.92 | 0.78 | 0.94 | 0.97 | 0.97 | 0.74 | 1.34 | - | - | - |

****Figures in parentheses are arc sine transformed values**

Present results are concurrent to the findings of Danilov (1976) who have also reported, 25°C as the optimum temperature for growth and development of Steinernematids and with special reference to *S. carpocapsae*. Similarly, Kung *et al*. (1991) from USA they have reported the survival of *S. carpocapsae* was significantly greater at lower temperatures (5-25°C) than at the highest temperature (35°C). The present findings also coincides with those of Ghally (1995) who also has mentioned that 20-25°C was the suitable temperature for the activity of *S. carpocapsae.*

**4.2.1.1.2 Effect of temperature on survival and mortality of *H. indica***

Results of the average effect of temperature on survival of the EPNs under laboratory conditions revealed that temperature of 25°C was the optimum temperature that showed maximum 99.95 per cent survival of the IJs of *H. indica*. With increase in temperature to 30°C and 35°C the percentage of survival 87.33 and 46.40 per cent respectively was observed. When temperature was decreased from 20°C, 15°C, 10°C, 5°C survival rates of IJs ranged from and 99.55, 96.61, 94.66 and 93.56 per cent respectively.

Data presented on survival of *H. indica* in the Table No. 4.3 showed that, the maximum mean survival 99.95 per cent of IJs was observed at 25°C from 24 to 96 hours of exposure. With increase in temperature to 30°C and 35°C the mean survival per cent of IJs reduced further significantly from 87.33 and 46.40 per cent.

As far as the percentage reduction in survival of the IJs was concerned, the data in Table No. 4.3 indicates, lesser decrease in survival with the decrease in temperature from 25°C, 20°C, 15°C, 10°C and 5°C as 0.05, 0.45, 3.39, 5.34 and 6.44 per cent as compared to increase in temperature from 25°C to 30°C and 35°C depicted a greater decrease of 0.05 to 12.67 and 53.60 per cent respectively.

Thus, from the above data, it can be concluded that *H. indica* showed less survival with increase in temperature as compared to decrease in temperature, which also justifies its presence in lesser number in hotter climatic conditions as of Chhattisgarh. The present findings also coincides with those of Ghally (1995) who also has mentioned that 20-25°C was the suitable temperature for the activity of *H. indica.*

**Table No. 4.3 Effect of temperature on mortality and survival percentage of *H. indica***

| Temp. | 24 hrs. | | 48 hrs. | | 72 hrs. | | 96 hrs. | | Mean | | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| °C | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Reduction in Survival |
| 5 °C | 3.75 | 96.25 | 6.30 | 93.70 | 7.62 | 92.37 | 8.05 | 91.95 | 6.43 | 93.56 | 6.44 |
| | (11.13) | (78.83) | (14.51) | (75.44) | (16.02) | (73.94) | (16.47) | (73.49) | | | |
| 10 °C | 3.90 | 96.10 | 4.77 | 95.22 | 6.30 | 93.70 | 6.37 | 93.62 | 5.33 | 94.66 | 5.34 |
| | (11.37) | (78.58) | (12.59) | (77.36) | (14.51) | (75.44) | (14.61) | (75.35) | | | |
| 15 °C | 1.82 | 98.17 | 2.77 | 97.22 | 3.25 | 96.75 | 5.67 | 72.82 | 3.38 | 96.61 | 3.39 |
| | (7.74) | (82.21) | (9.56) | (80.40) | (10.34) | (79.62) | (13.76) | (61.30) | | | |
| 20 °C | 0.00 | 100.00 | 0.00 | 100.00 | 0.37 | 99.62 | 1.40 | 98.60 | 0.44 | 99.55 | 0.45 |
| | (0.12) | (90.00) | (0.12) | (90.00) | (2.47) | (87.51) | (6.74) | (83.22) | | | |
| 25 °C | 0.00 | 100.00 | 0.00 | 100.00 | 0.00 | 100.00 | 0.20 | 99.80 | 0.05 | 99.95 | 0.05 |
| | (0.12) | (90.00) | (0.12) | (90.00) | (0.12) | (90.00) | (1.79) | (88.18) | | | |
| 30 °C | 6.30 | 93.70 | 9.25 | 90.75 | 15.25 | 84.75 | 19.87 | 80.12 | 12.66 | 87.33 | 12.67 |
| | (14.51) | (75.44) | (17.69) | (72.27) | (22.97) | (66.99) | (26.46) | (63.50) | | | |
| 35 °C | 38.02 | 61.97 | 45.75 | 54.25 | 56.75 | 43.25 | 73.85 | 26.15 | 53.59 | 46.40 | 53.60 |
| | (38.05) | (51.90) | (42.54) | (47.41) | (48.86) | (41.10) | (59.22) | (30.74) | | | |
| Control | 0.00 | 100.00 | 0.00 | 100.00 | 0.00 | 100.00 | 0.00 | 100.00 | 0.00 | 100.00 | - |
| (25 ±2°C) | (1.00) | (90.00) | (1.00) | (90.00) | (1.00) | (90.00) | (1.00) | (90.00) | | | |
| SE(m) | 0.27 | 0.27 | 0.28 | 0.28 | 0.58 | 0.58 | 0.46 | 0.46 | - | - | - |
| C.D.(1%) | 0.81 | 0.81 | 0.82 | 0.82 | 1.70 | 1.70 | 1.36 | 1.36 | - | - | - |

****Figures in parentheses are arc sine transformed values**

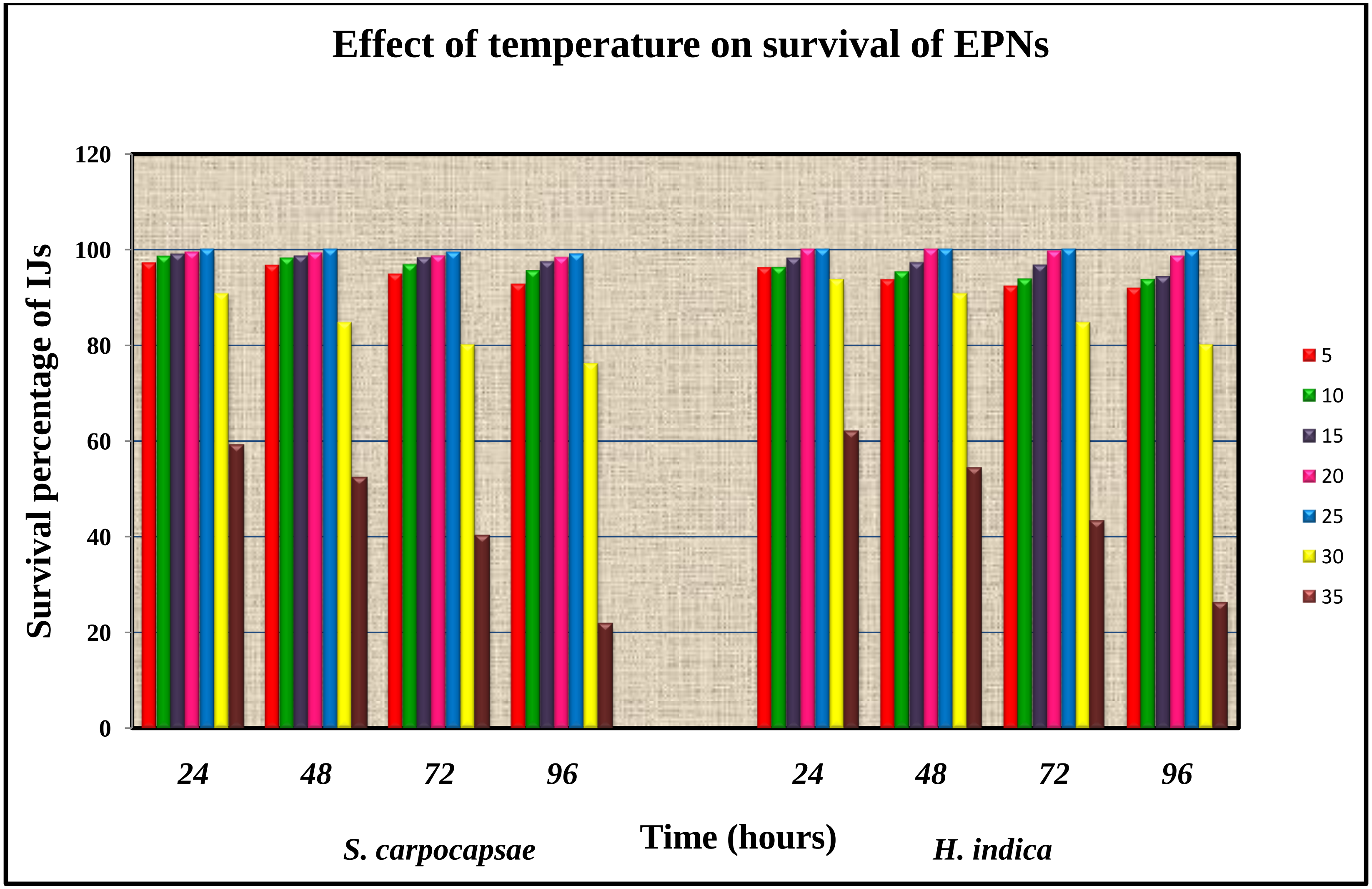



**Fig.4.1 Effect of temperature on percent survival of EPNs at different duration.**

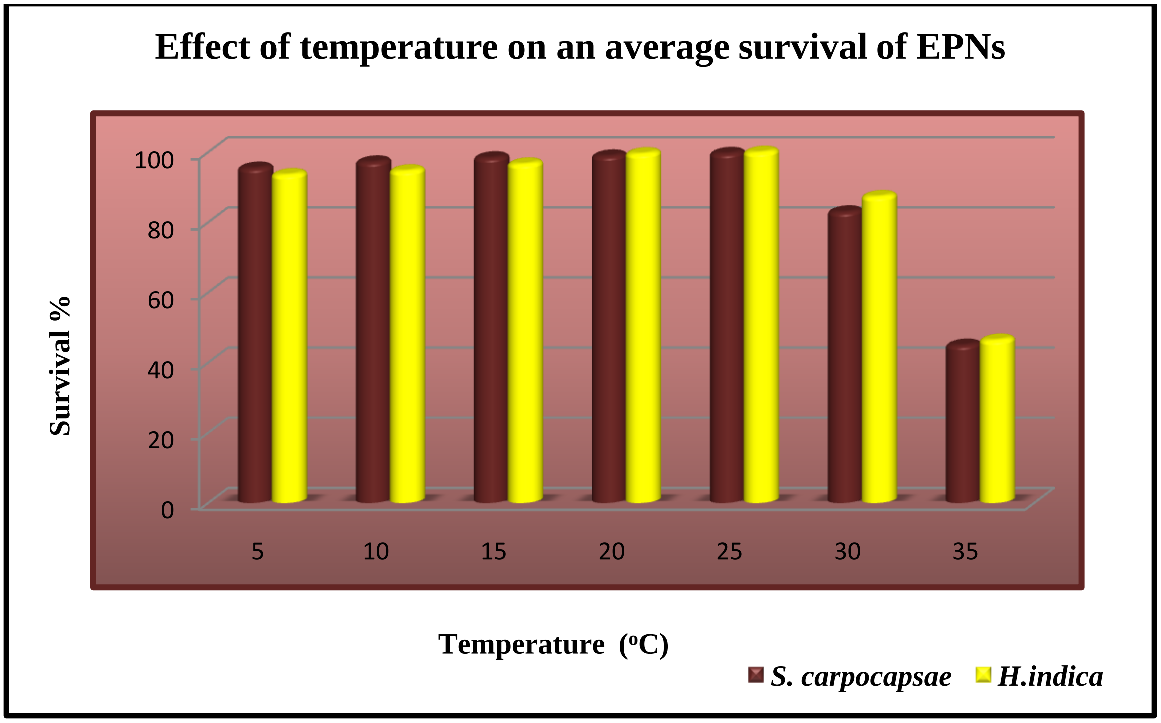



**Fig.4.2 Effect of temperature on mean percent survival of EPNs**

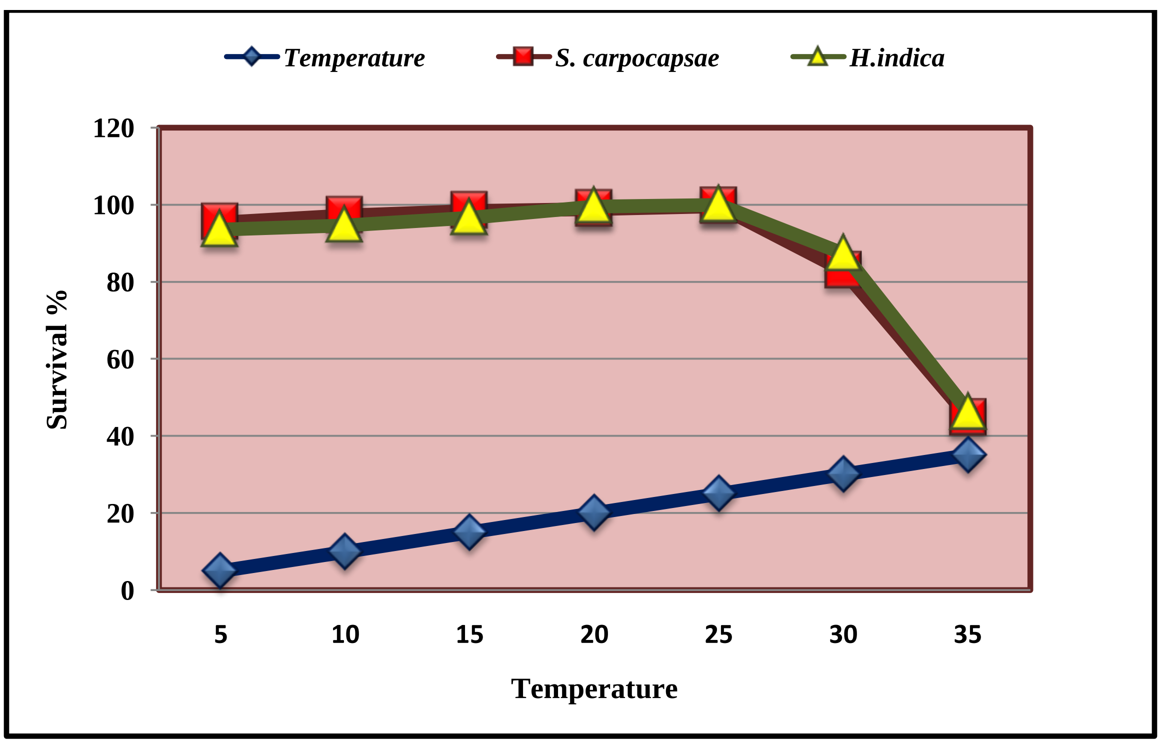



**Fig.4.3 Correlation coffecient value of mean percent survival of EPNs with temperature**

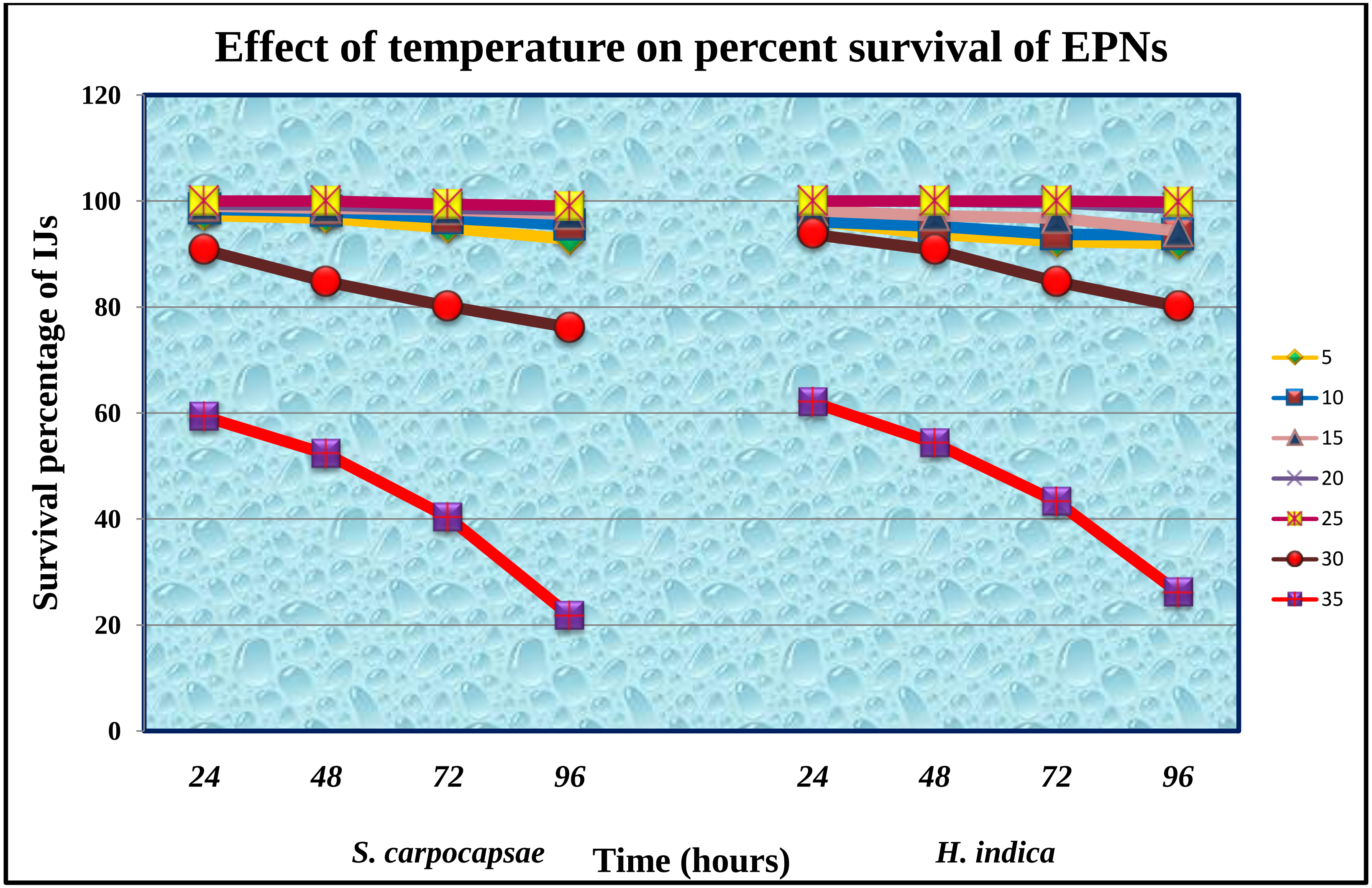



**Fig.4.4 Correlation of temperature with percent survival of EPNs**

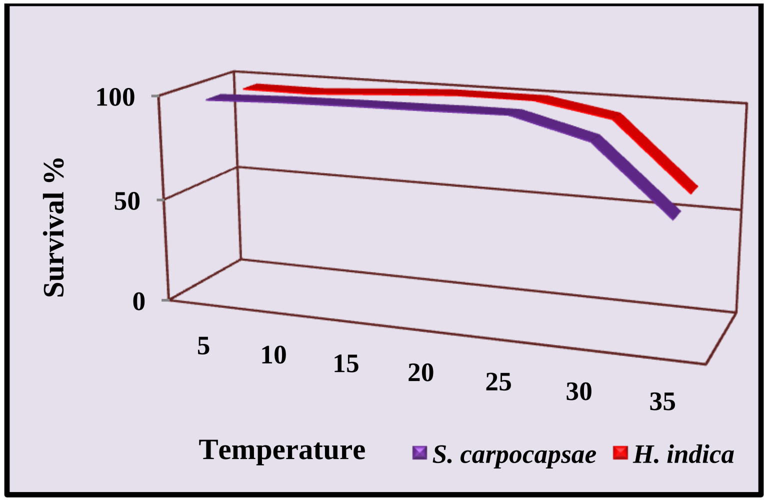



**Fig.4.5 Effect of temperature on survival of EPNs in 24 hour**

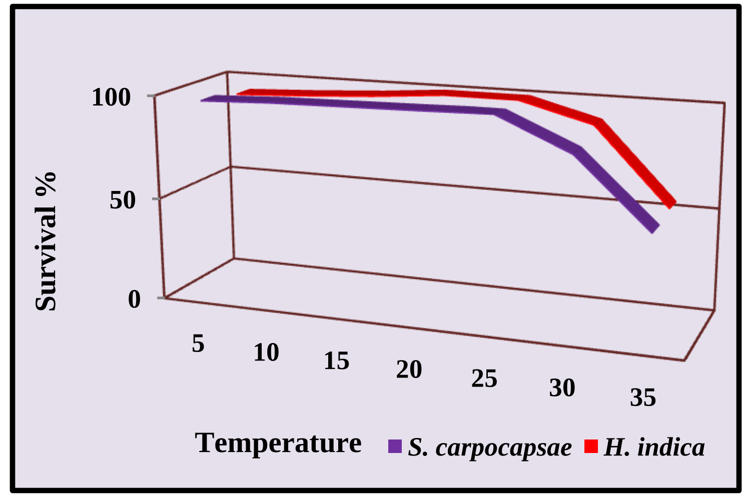



**Fig.4.6 Effect of temperature on survival of EPNs in 48 hours**

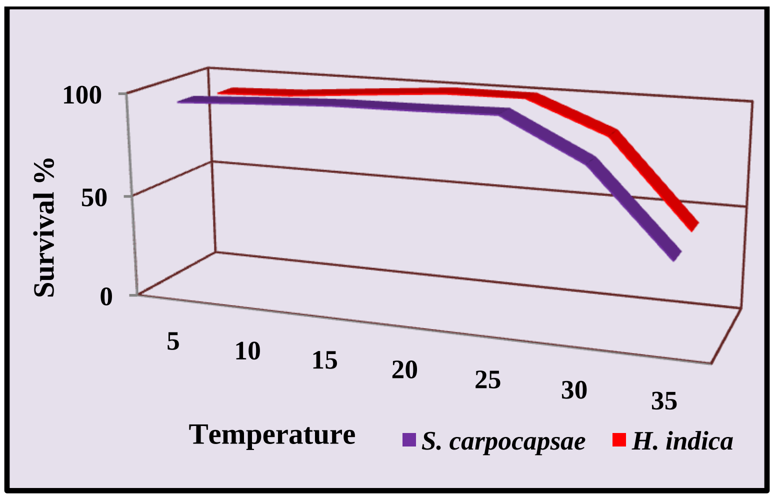



**Fig.4.7 Effect of temperature on survival of EPNs in 72 hours**

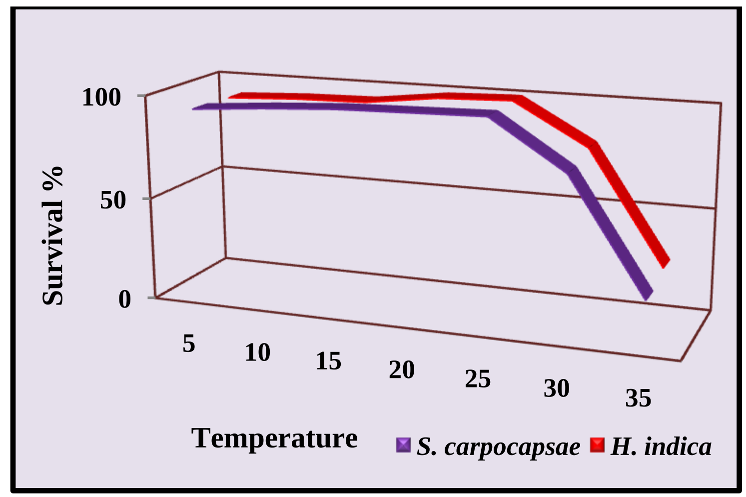



**Fig.4.8 Effect of temperature on survival of EPNs in 96 hours**

### 4.2.2. Effect of U.V radiation on EPNs:-

#### 4.2.2.1 Effect of U.V radiation on the survival and mortality of EPNs

The effect of U.V radiation on survival and mortality of *S. carpocapsae* and *H. indica* presented in the Table No. 4.4 with the respect of time of exposure to U.V radiation *i.e.*15.00, 30.00, 45.00, 60.00, 75.00 and 90.00 minutes time intervals revealed that the IJs of both *S. carpocapsae* and *H. indica* exposed to U.V. radiation for a longer period, resulted in the mortality of IJs.

#### 4.2.2.1.1 Effect of U.V radiation on the survival and mortality of *S. carpocapsae*

Observations recorded on the effect of U.V radiation on survival of *S. carpocapsae* presented in the Table No. 4.4 revealed that significant maximum survival of 86.12 per cent of IJs in the treatment of 15.00 minutes of exposure (254 nm) of U.V radiation and minimum 0.00 per cent survival were recorded in 120.00 minutes of exposure. Significant reduction in the percentage of survival from 75.00, 65.87, 54.25, 45.12, 32.25 and 15.50 was depicted on increasing the duration of exposure from 30.00, 45.00, 60.00, 75.00, 90.00 and 105.00 minutes respectively. 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. 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.

#### 4.2.2.1.2 Effect of U.V Radiation on the survival and mortality of *H. indica*.

Observations recorded on the effect of U.V radiation on survival of *H. indica* presented in the Table No. 4.4 revealed that significant maximum survival of 82.37 per cent of IJs was recorded in the treatment of 15.00 minutes of exposure (254 nm) of U.V radiation and minimum 0.00 per cent survival was recorded in 120.00 minutes of exposure. Significant reduction in the percentage of survival from 64.25, 44.25, 29.00, 16.75 and 9.25 was depicted on increasing the duration of exposure from 30.00, 45.00, 60.00, 75.00 and 90.00 minutes respectively. In the present study, results pertaining to the effect of U.V. radiation on nematode survival revealed that, in case of *H. indica* absolute mortality was recorded after 105 minutes of exposure to U.V. radiation at 254 nm wave length.

In general, it was observed that when the IJs either *S. carpocapsae* or *H. indica* were exposed to U.V. radiation for a longer period, it could result in complete death of the IJs due to affecting their activity. Finally, it could be concluded from the results that among the two EPNs, *H. indica* was found to be more sensitive to U.V radiation than *S. carpocapsae* this could result in 100.00 per cent mortality after 105 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 No. 4.4 Effect of UV radiation on percent survival and mortality of IJs**

| **Treatment** | ***S. Carpocapsae*** | | ***H. indica*** | |
|---|---|---|---|---|
| | **Mortality %** | **Survival %** | **Mortality %** | **Survival %** |
| **15.00 min.** | 13.87<br>(21.85) | 86.12<br>(68.10) | 17.62<br>(24.81) | 82.37<br>(65.15) |
| **30.00 min.** | 25.00<br>(29.98) | 75.00<br>(59.97) | 35.75<br>(36.70) | 64.25<br>(53.25) |
| **45.00 min.** | 34.12<br>(35.72) | 65.87<br>(54.23) | 55.75<br>(48.28) | 44.25<br>(41.68) |
| **60.00 min.** | 45.75<br>(42.54) | 54.25<br>(47.41) | 71.00<br>(57.39) | 29.00<br>(32.56) |
| **75.00 min.** | 54.87<br>(47.77) | 45.12<br>(42.18) | 83.25<br>(65.81) | 16.75<br>(24.14) |
| **90.00 min.** | 67.75<br>(55.37) | 32.25<br>(34.58) | 90.75<br>(72.27) | 9.25<br>(17.69) |
| **105.00 min.** | 84.50<br>(66.79) | 15.50<br>(23.16) | 100.00<br>(90.00) | 0.00<br>(1.00) |
| **120.00 min.** | 100.00<br>(90.00) | 0.00<br>(0.12) | 100.00<br>(90.00) | 0.00<br>(0.12) |
| **Control<br>(No exposure)** | 0.00<br>(1.00) | 100.00<br>(90.00) | 0.00<br>(1.00) | 100.00<br>(90.00) |
| **SE(m)** | 0.21 | 0.21 | 0.21 | 0.21 |
| **C.D.(1%)** | 0.63 | 0.63 | 0.61 | 0.61 |

**** Figures in parentheses are arc sine transformed value**

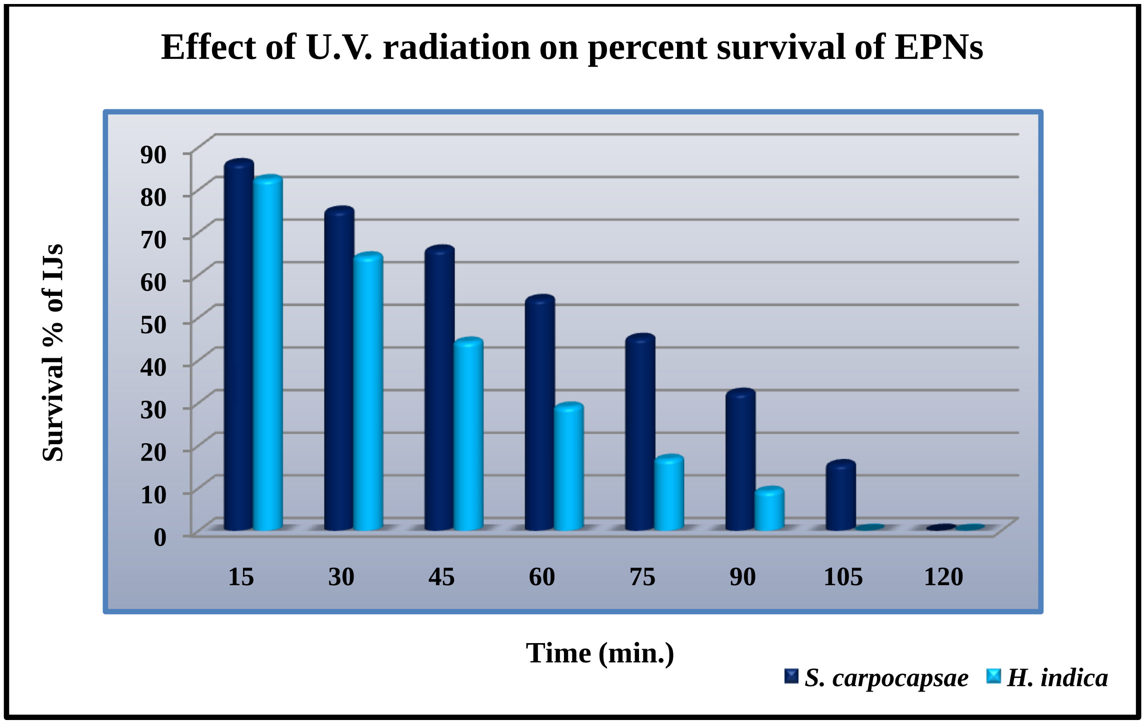



**Fig.4.9 Effect of U.V. radiation on percent survival of EPNs**

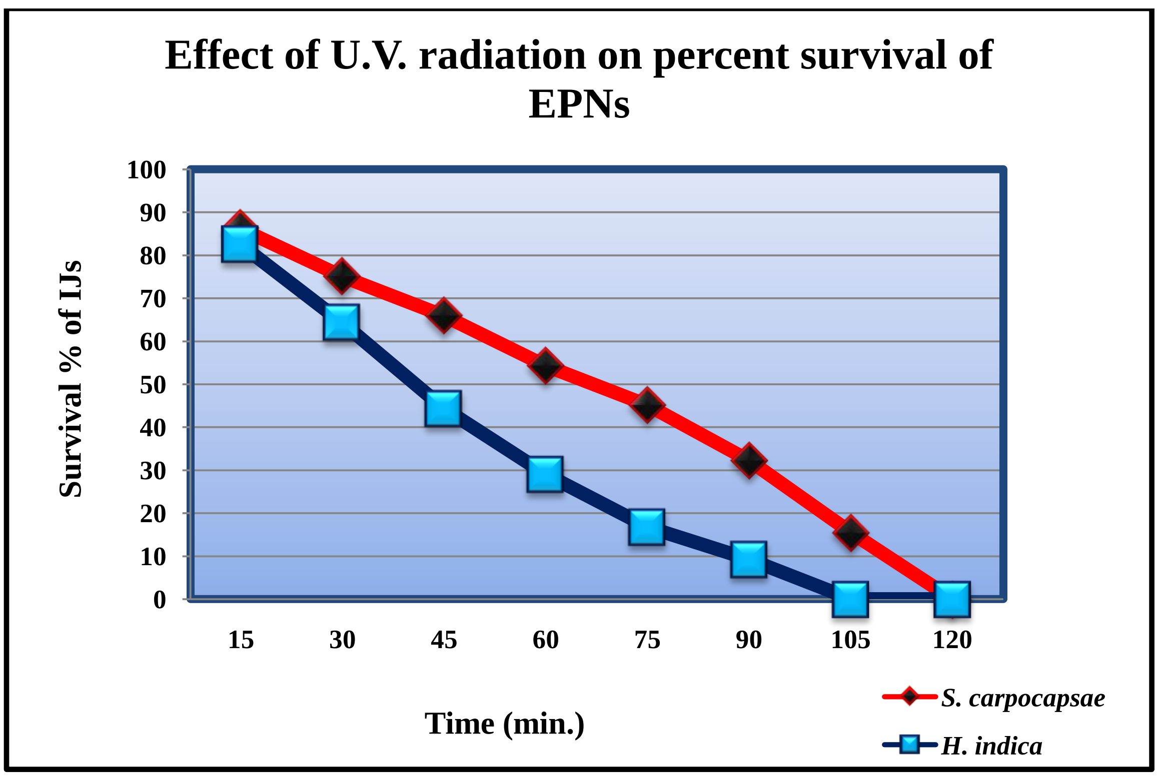



**Fig.4.10 Correlation of U.V. radiation on percent survival of EPNs**

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. Similar observations were made by Gulsar Banu and Rajendran (2003) they have also recorded absolute mortality of the infective juveniles, 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.

**4.2.2.2 Effect of U.V radiation on pathogenicity of EPNs on *G. mellonella***

Experimental results pertaining to the effect of U.V radiation on pathogenicity of *S. carpocapsae* and *H. indica* on *G. mellonella* larva presented in Table No. 4.5.

**4.2.2.2.1 Effect of U.V radiation on pathogenicity of *S. carpocapsae* on *G. mellonella***

Experimental results pertaining to the effect of U.V radiation on pathogenicity of *S. carpocapsae* on *G. mellonella* larvae presented in Table No. 4.5 revealed that, *S. carpocapsae* was more virulent in causing mortality of the test insect, even after exposure to U.V. radiation more for 75.00 minutes able to infect *G. mellonella* larva upto 25.75 per cent. Exposure for a minimum period of 15.00 minutes to U.V. radiation, IJs of *S. carpocapsae* caused 100.00 per cent infection in the test insect. If the IJs were exposed to U.V. radiation for 30.00 minutes, it was able to cause result 88.87 per cent mortality, followed by 75.75 per cent after 45.00 minutes, 41.75 after 60.00 minutes and 25.75 per cent after as of 75.00 minutes to U.V radiation. Hence, from the above results it can be concluded that there was significant effect of U.V radiation with respect of duration of exposure on the pathogenicity of IJs of *S. carpocapsae.*

**4.2.2.2.2 Effect of U.V radiation on pathogenicity of *H. indica* on *G. mellonella***

Experimental results pertaining to the effect of U.V radiation on pathogenicity of *H. indica* on *G. mellonella* larvae presented in Table No. 4.5 revealed that, *H. indica* also exhibited 100.00 per cent pathogenicity in the test insect larvae of *G. mellonella* of an exposure of 15.00 minutes as in *S. carpocapsae.* Exposure of IJs for 30.00 minutes to

U.V. radiation resulted in 77.75 per cent mortality followed by 55.75 per cent after 45.00 minutes and 30.75 per cent after 60.00 minutes of exposure. *H. indica* could not infect the *G. mellonella* larvae, when the IJs were exposed for more than 60.00 minutes to U.V radiation. Thus, it can be concluded from the above results that there was significant decrease in pathogenicity of IJs of *H. indica* with respect of increase in the duration of exposure to U.V. radiation.

Comparing the pathogenicity of the two species of IJs, it can be stated that the IJs of *S. carpocapsae* were sturdier and could sustain longer period of exposure to U.V. radiation.

**Table No. 4.5 Effect of UV radiation on pathogenicity of EPNs on *G. mellonella***

| Time | Mortality percentage of larvae | |
|---|---|---|
| | ***S. Carpocapsae*** | ***H. indica*** |
| **15.00 min.** | 100.00 | 100.00 |
| | (90.00) | (90.00) |
| **30.00 min.** | 88.87 | 77.75 |
| | (70.49) | (61.83) |
| **45.00 min.** | 75.75 | 55.75 |
| | (60.47) | (48.28) |
| **60.00 min.** | 41.75 | 30.75 |
| | (40.23) | (33.66) |
| **75.00 min.** | 25.75 | 0.00 |
| | (30.48) | (0.12) |
| **90.00 min.** | 0.00 | 0.00 |
| | (0.12) | (0.12) |
| **105.00 min.** | 0.00 | 0.00 |
| | (0.12) | (0.12) |
| **120.00 min.** | 0.00 | 0.00 |
| | (0.12) | (0.12) |
| **Control** | 0.00 | 0.00 |
| | (0.12) | (0.12) |
| **SE(m)** | 0.17 | 0.11 |
| **C.D. (1%)** | 0.51 | 0.34 |

****Figures in parentheses are arc sine transformed values.**

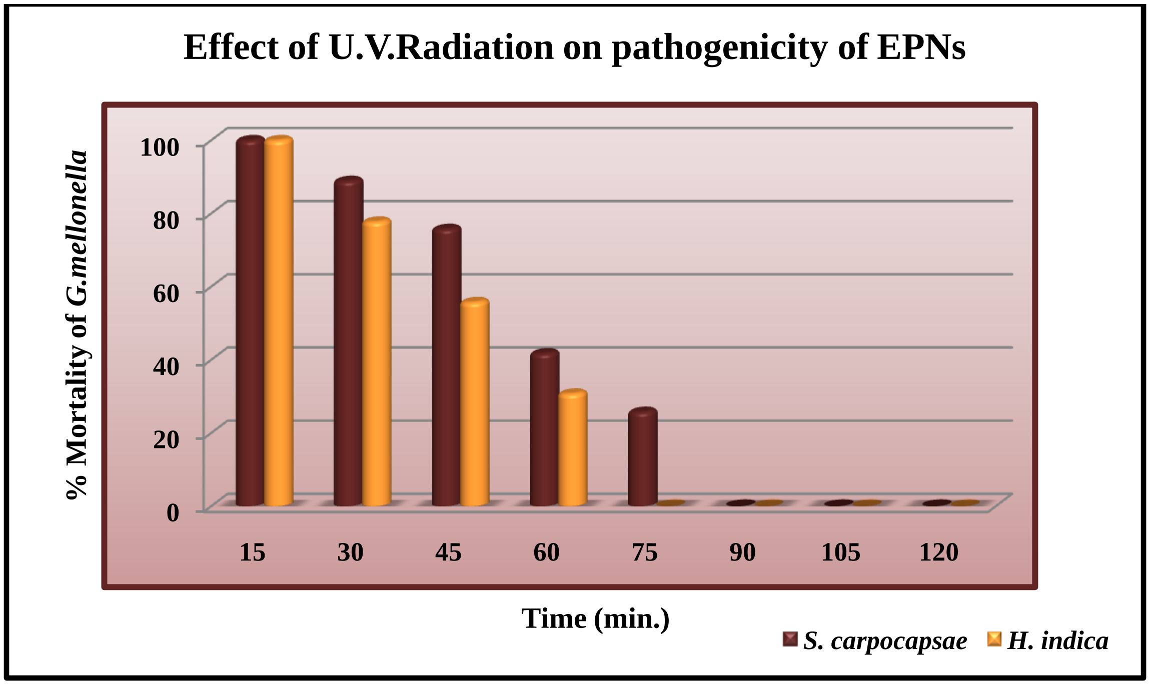



**Fig.4.11 Effect of U.V. radiation on pathogenicity of EPNs**

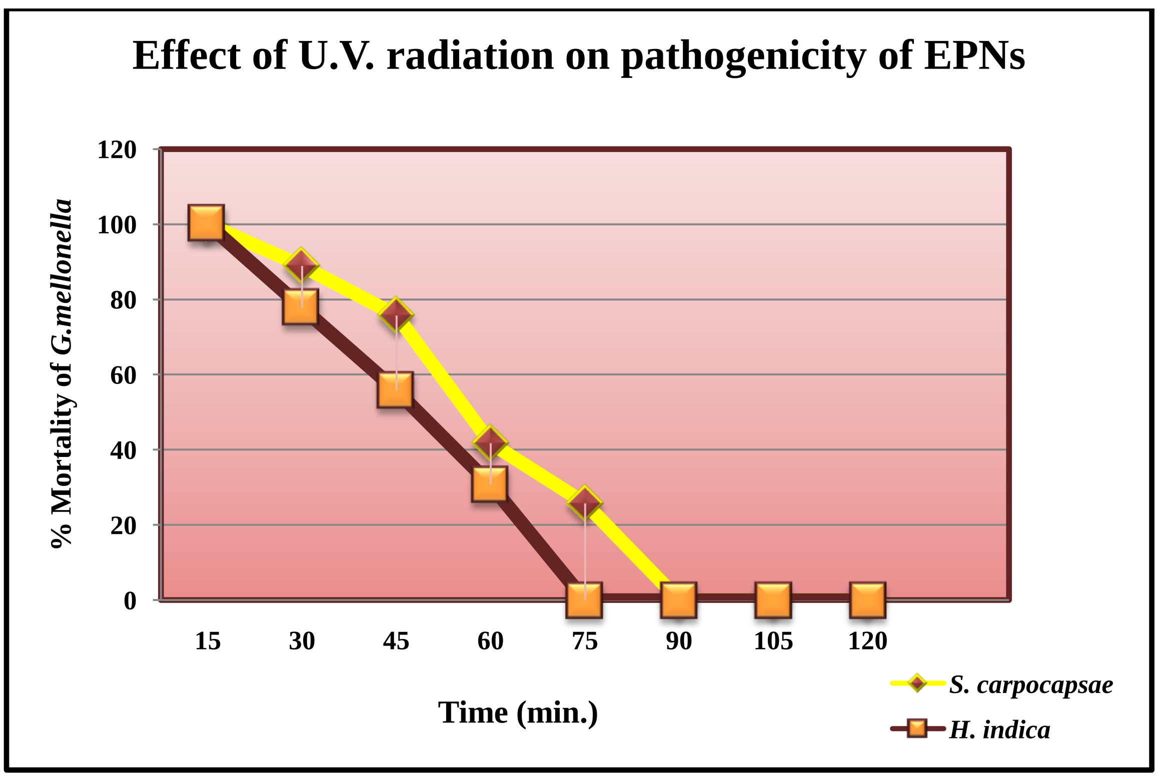



**Fig.4.12 Correlation of U.V. radiation on pathogenicity of EPNs**

### 4.2.3 Effect of pH on EPNs

Experiments conducted for observing the influence of different levels of pH *i.e.* 4.00, 5.00, 6.00, 7.00, 8.00 and 9.00 on survival and mortality of the IJs of the EPNs of the two species *i.e. S. carpocapsae* and *H. indica,* are presented in the Table No. 4.6 and 4.7.

#### 4.2.3.1 Effect of pH on survival and mortality of IJs of *S. carpocapsae*

Data regarding the mean percentage survival of the IJs of *S. carpocapsae* at different pH presented in Table No. 4.6 revealed that there was a significant gradual increase in percentage survival of IJs with increasing pH from 4.00 to 9.00. Hundered per cent survival of the IJs of *S. carpocapsae* was recorded after seven days in all the different values of pH tested and there were no differences among the treatments. But as the duration of exposure to different levels of pH increased, the percent of survival decreased. Optimum level of pH was found to be at pH 7.00 *i.e.* neutral in which significant maximum mean survival of 99.31 per cent was recorded after 28 days. Lesser values of pH than neutral *i.e.* 6.00, 5.00and 4.00 towards acidic depicted a decline in the survival of IJs from 98.50, 97.50 and 96.43 per cent respectively. Similarly, increase in pH from the neutral value to 8.00 and 9.00 *i.e.* towards alkaline also exhibited lesser survival of 98.75 and 98.93as compared to 99.31 in the pH value of 7.00

#### 4.2.3.2 Effect of pH on survival and mortality of *H. indica.*

Data regarding the mean percentage survival of the IJs of *H. indica* at different pH presented in Table No. 4.7 revealed that there was a significant gradual decrease in percentage survival of IJs of *H. indica* with increasing pH from 5.00 to 9.00. Highest percent survival in the IJs of *H. indica* was recorded of 95.00 per cent at pH 5.00 and lowest 69.00 at pH 4.00 after seven days. But, as the duration of exposure to different levels of pH increased, the percent of survival decreased. Optimum level of pH was found to be 5.00 (acidic) in which significant maximum mean survival of 88.81 per cent was recorded after 28 days. With increasing the pH further from acidic to neutral *i.e.* 6.00 and 7.00 declines in the survival of IJs of *H. indica* from 85.50 and 81.93 per cent respectively was recorded. Similarly, increase in pH from neutral value i.e. from 7.00 to 8.00 and 9.00 *i.e.* towards alkaline also exhibited significant lesser survival of 74.81 and 67.12 as compared to 88.81 in the pH value of 5.00.

**Table No. 4.6 Effect of pH on mortality and survival percentage of *S. carpocapsae***

| pH | 7 Days | | 14 Days | | 21 Days | | 28 Days | | Mean | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Survival %** | **Mortality %** | **Survival %** | **Mortality %** | **Survival %** | **Mortality %** | **Survival %** | **Mortality %** | **Survival %** | **Mortality %** |
| **4** | 100.00 | 0.00 | 97.75 | 2.25 | 94.50 | 5.50 | 93.50 | 6.50 | 96.43 | 3.56 |
| | (90.00) | (0.01) | (81.37) | (8.58) | (76.41) | (13.54) | (75.20) | (14.75) | | |
| **5** | 100.00 | 0.00 | 98.75 | 1.25 | 96.25 | 3.75 | 95.00 | 5.00 | 97.50 | 2.50 |
| | (90.00) | (0.01) | (83.62) | (6.33) | (78.82) | (11.14) | (77.08) | (12.88) | | |
| **6** | 100.00 | 0.00 | 99.50 | 0.50 | 97.50 | 2.50 | 97.00 | 3.00 | 98.50 | 1.50 |
| | (90.00) | (0.01) | (87.11) | (2.86) | (80.91) | (9.04) | (80.06) | (9.90) | | |
| **7** | 100.00 | 0.00 | 100.00 | 0.00 | 99.25 | 0.75 | 98.00 | 2.00 | 99.31 | 0.68 |
| | (90.00) | (0.01) | (90.00) | (1.00) | (85.67) | (4.30) | (81.97) | (7.99) | | |
| **8** | 100.00 | 0.00 | 100.00 | 0.00 | 98.50 | 1.50 | 96.50 | 3.50 | 98.75 | 1.25 |
| | (90.00) | (0.01) | (90.00) | (1.00) | (83.03) | (6.93) | (79.21) | (10.75) | | |
| **9** | 100.00 | 0.00 | 100.00 | 0.00 | 98.25 | 1.75 | 97.50 | 2.50 | 98.93 | 1.06 |
| | (90.00) | (0.01) | (90.00) | (1.00) | (82.43) | (7.52) | (80.91) | (6.93) | | |
| **SE(m)** | - | - | 0.74 | 0.74 | 0.76 | 0.75 | 0.62 | 0.62 | - | - |
| **C.D.(1%)** | - | - | 2.23 | 2.22 | 2.28 | 2.27 | 1.86 | 1.86 | - | - |

****Figures in parentheses are arc sine transformed values**

**Table No. 4.7 Effect of pH on mortality and survival percentage of *H. indica***

| pH | 7 Days | | 14 Days | | 21 Days | | 28 Days | | Mean | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Survival % | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Mortality % | Survival % | Mortality % |
| **4** | 69.00 | 31.00 | 65.75 | 34.25 | 61.25 | 38.75 | 52.50 | 47.50 | 62.12 | 37.87 |
| | (56.14) | (33.81) | (54.16) | (35.80) | (51.48) | (38.48) | (46.41) | (43.54) | | |
| **5** | 95.00 | 5.00 | 92.50 | 7.50 | 86.75 | 13.25 | 81.00 | 19.00 | 88.81 | 11.18 |
| | (77.08) | (12.88) | (74.08) | (15.87) | (68.63) | (21.32) | (64.13) | (25.82) | | |
| **6** | 92.25 | 7.75 | 89.50 | 10.50 | 82.00 | 18.00 | 78.25 | 21.75 | 85.50 | 14.50 |
| | (73.82) | (16.13) | (71.08) | (18.87) | (64.87) | (25.09) | (62.18) | (27.78) | | |
| **7** | 86.50 | 13.50 | 86.50 | 13.50 | 79.00 | 21.00 | 75.75 | 24.25 | 81.93 | 18.06 |
| | (68.43) | (21.53) | (68.43) | (21.53) | (62.70) | (27.26) | (60.47) | (29.48) | | |
| **8** | 83.75 | 16.25 | 78.25 | 21.75 | 73.25 | 26.75 | 64.00 | 36.00 | 74.81 | 25.18 |
| | (60.98) | (23.75) | (62.20) | (27.75) | (58.83) | (31.13) | (53.11) | (36.85) | | |
| **9** | 76.50 | 23.50 | 70.00 | 30.00 | 65.00 | 35.00 | 57.00 | 43.00 | 67.12 | 32.87 |
| | (60.98) | (28.97) | (56.76) | (33.19) | (53.70) | (36.25) | (49.00) | (40.95) | | |
| **SE(m)** | 0.45 | 0.45 | 0.55 | 0.55 | 0.29 | 0.29 | 0.30 | 0.30 | - | - |
| **C.D.(1%)** | 1.35 | 1.35 | 1.64 | 1.64 | 0.88 | 0.88 | 0.91 | 0.91 | - | - |

****Figures in parentheses are arc sine transformed values**

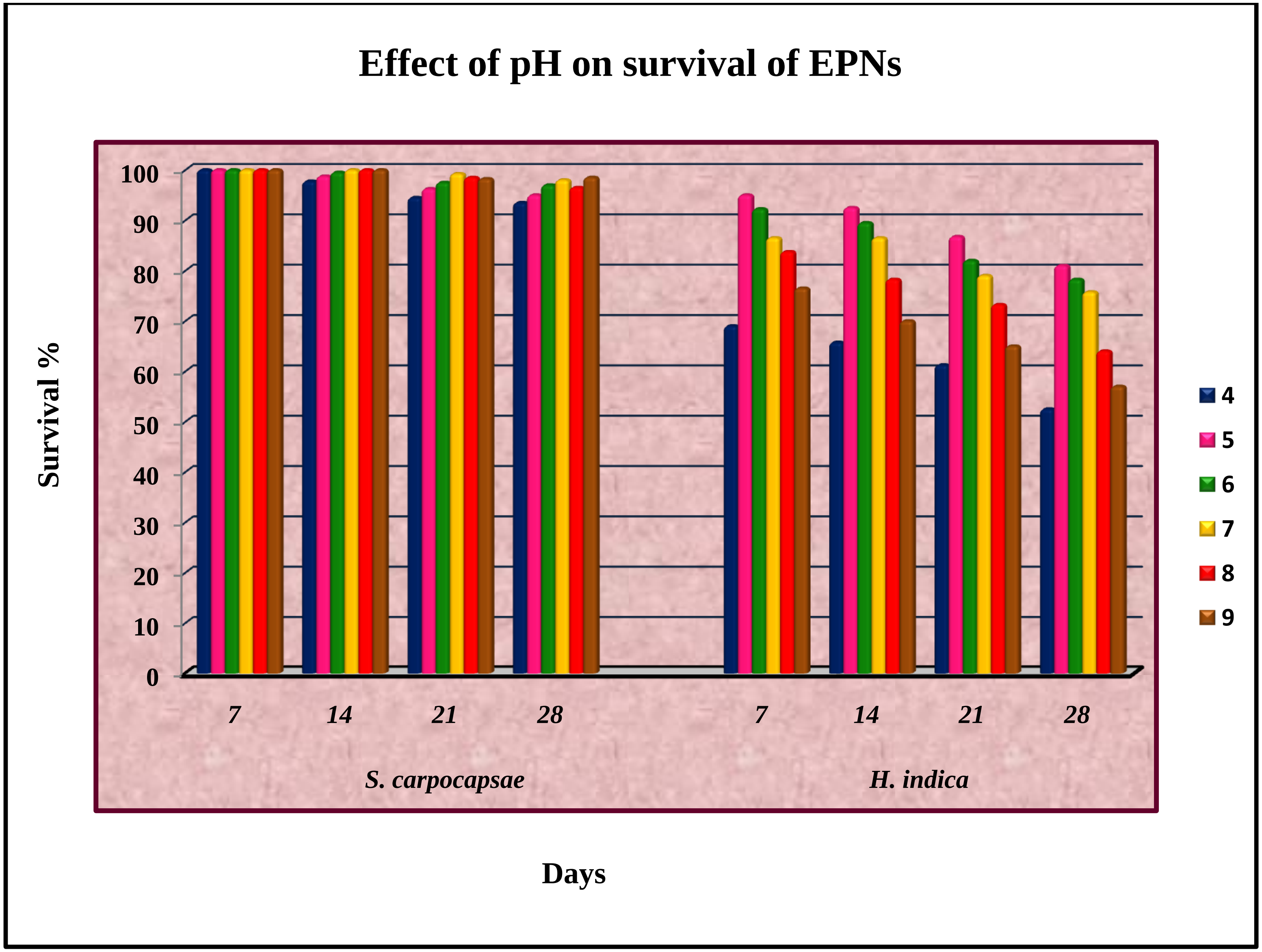



**Fig.4.13 Effect of pH on survival of EPNs at different duration**

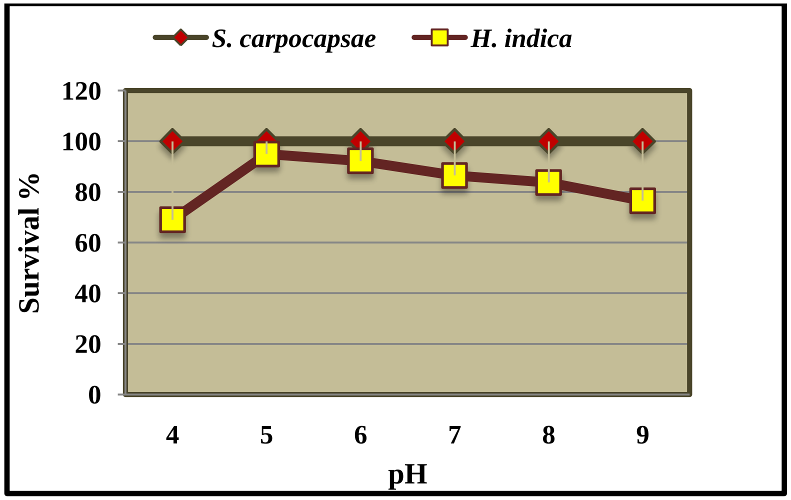



**Fig.4.14 Effect of pH on survival of EPNs in 24 hours**

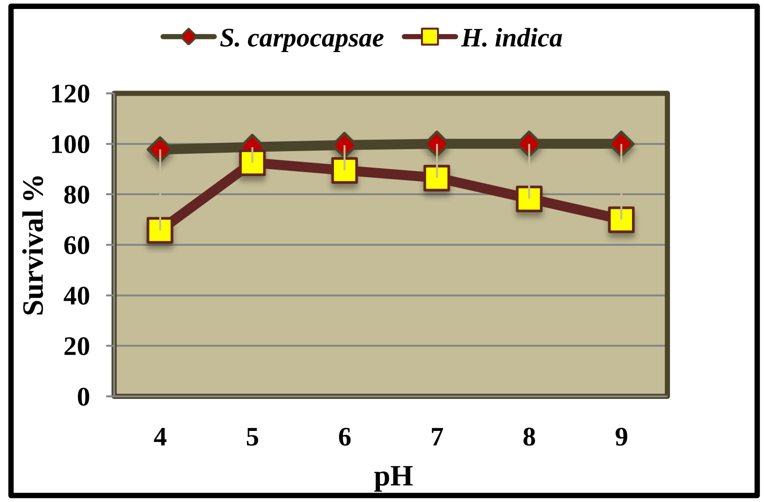



**Fig.4.15 Effect of pH on survival of EPNs in 48 hours**

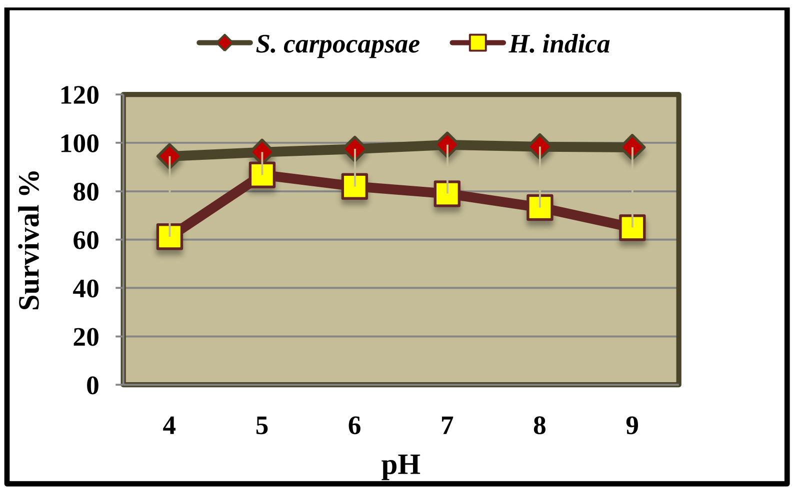



**Fig.4.16 Effect of pH on survival of EPNs in 72 hours**

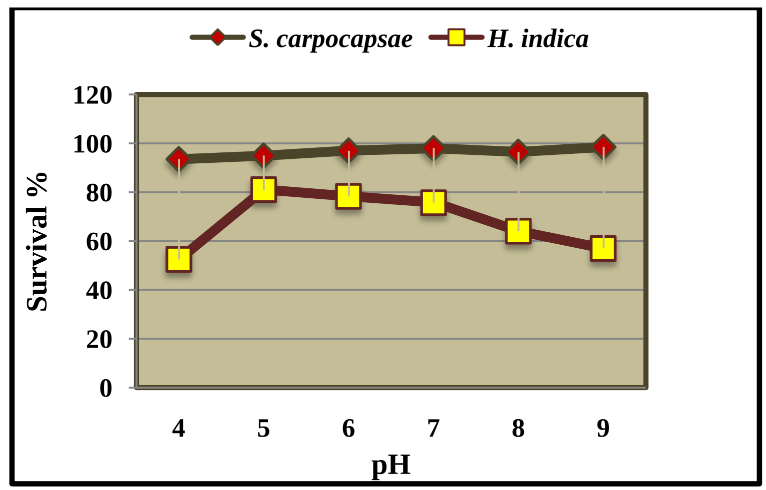



**Fig.4.17 Effect of pH on survival of EPNs in 96 hours**

Thus, from the above findings, it can be concluded that pH of 7.00 (neutral )was the most suitable level for mean maximum survival of the *S. carpocapsae* while pH level of 5.00 was found to best exhibiting mean maximum survival of 88.81 per cent for *H. indica*. This findings are in agreement with Similar results were reported by Kung *et al.* (1990) who observed lowest survival of the IJs of the two nematode species *i.e. S. carpocapsae* and *S. glaseri* at acidic pH of 5.00 and basic pH of 10.00 and further confirms the reports made by Cheng and Hou (1997) who also observed low nematode survival under high acidic conditions.

### 4.2.4 Effect of soil depth and types of soils on vertical movement of EPNs

Results pertaining to the experiment conducted on vertical movement and pathogenicity of *S. carpocapsae* and *H. indica* on different soil type such as sandy, sandy loam and clay soils with various depths *i.e.* 5.00, 10.00, 15.00, 20.00, 25.00, 30.00 and 35.00.

#### 4.2.4.1 Effect of soil depth on vertical movement of *S. carpocapsae* in sandy soil

Studies conducted to record the vertical movement of *S. carpocapsae* at different depths in sandy soil at duration of time as indicated by the mortality of the larvae of *G. mellonella* was analysed in CRD (Completely randomized design) replicated three times from the data, it is clear that as the depth of the soil increased, resulted in decreased mortality of the test insects, *G. mellonella* larvae in the species of EPNs tested.

Maximum mortality of the larvae was observed at the depth of 5.00 cms. Observations recorded two days after inoculation of 1000 IJs, depicted 33.33 per cent mortality on 2$^{nd}$ day which increased to 75.00, 83.33 and 100.00 per cent after 4$^{th}$, 6$^{th}$ and 8$^{th}$ days after treatment respectively.

At 10.00 cms depth no mortality was recorded on the 2$^{nd}$ day and from 4$^{th}$ day mortality of 33.33 per cent was observed, which increased to 41.66, 66.66 and 91.66 per cent after 6$^{th}$, 8$^{th}$ and 10$^{th}$ days after treatment respectively.

At 15.00 cms depth no mortality was recorded on the 2$^{nd}$, 4$^{th}$ and 6$^{th}$ days after treatment and mortality observed was from 8$^{th}$ and 10$^{th}$ days as 16.66 and 33.33 per cent respectively. At depth of more than 15.00 cms *i.e.* 20.00, 25.00, 30.00 and 35.00 cms no mortality 0.00 per cent was recorded.

**Table No. 4.8 Vertical movement of *S. carpocapsae* at different depths (cms) and duration of time (days) in sandy soil**

| Treatment | Percent mortality of *G. mellonella* larvae in sandy soil at different time interval (days) | | | | | |
|---|---|---|---|---|---|---|
| | **2 days** | **4 days** | **6 days** | **8 days** | **10 days** | **Mean** |
| **5.00 cm** | 33.33 (34.98) | 75.00 (64.98) | 83.33 (69.98) | 100.00 (90.00) | 100.00 (90.00) | 78.33 |
| **10.00 cm** | 0.00 (0.06) | 33.33 (34.98) | 41.66 (39.98) | 66.66 (54.97) | 91.66 (79.99) | 46.66 |
| **15.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 16.66 (19.99) | 33.33 (34.98) | 9.99 |
| **20.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **25.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **30.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **35.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **Control** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **SE(m)** | 1.76 | 5.00 | 3.95 | 3.95 | 3.95 | - |
| **C.D. (1%)** | 5.34 | 15.12 | 11.95 | 11.94 | 11.95 | - |

****Figures in parentheses are arc sine transformed value**

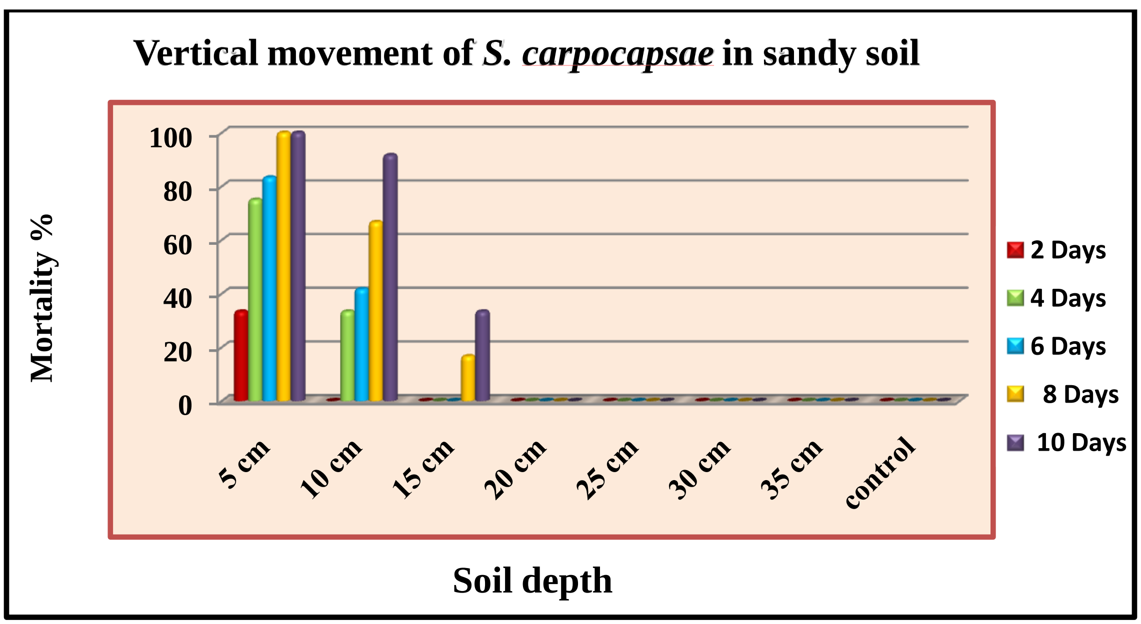



**Fig.4.18 Vertical movement of *S. carpocapsae* in sandy soil**

#### 4.2.4.2 Effect of soil depth on vertical movement of *S. carpocapsae* in sandy loam soil

Studies conducted to record the vertical movement of *S. carpocapsae* at different depths and duration of time in sandy loam soil as indicated by the mortality of the larvae of *G. mellonella* was analysed in CRD (Completely randomized design) replicated three times from the data, it is clear that as the depth of the soil increased, resulted in decreased mortality of the test insects, *G. mellonella* larvae in the species of EPNs tested.

**Table No.4.9 Vertical movement of *S. carpocapsae* at different depths (cms) and duration of time (days) in sandy loam soil**

| Treatment | Percent mortality of *G. mellonella* larvae in sandy loam soil at different time interval (days) | | | | | |
|---|---|---|---|---|---|---|
| | **2 days** | **4 days** | **6 days** | **8 days** | **10 days** | **Mean** |
| **5.00 cm** | 66.66 (54.97) | 83.33 (69.98) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 89.99 |
| **10.00 cm** | 33.33 (34.98) | 66.66 (54.97) | 91.66 (79.99) | 100.00 (90.00) | 100.00 (90.00) | 78.33 |
| **15.00 cm** | 0.00 (0.06) | 25.00 (29.98) | 33.33 (34.98) | 58.33 (49.98) | 83.33 (69.98) | 39.99 |
| **20.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 25.00 (29.98) | 50.00 (44.98) | 15.00 |
| **25.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **30.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **35.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **Control** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **SE(m)** | 2.49 | 3.95 | 3.95 | 1.76 | 3.53 | - |
| **C.D. (1%)** | 7.55 | 11.95 | 11.95 | 5.34 | 10.69 | - |

****Figures in parentheses are arc sine transformed value**

Maximum mortality of the larvae was observed at the depth of 5.00 cms. Observations recorded two days after inoculation of 1000 IJs, depicted 66.66 per cent mortality on 2$^{nd}$ day which increased to 83.33 and 100.00 per cent after 4$^{th}$ and 6$^{th}$ days after treatment respectively.

At 10.00 cms depth 33.33 per cent mortality was recorded on the 2nd day which increased to 66.66, 91.66 and 100.00 per cent after 4th, 6th days and 8th days after treatment respectively.

At 15.00 cms depth no mortality was recorded on the 2nd and from 4th days mortality of 25.00 per cent was observed, which increased to 33.33, 58.33 and 83.33 per cent after 6th, 8th and 10th days treatment respectively.

At 20.00 cms depth no mortality was recorded on the 2nd day and from 4th day and from 8th day mortality of 25.00 per cent was observed, which increased to 50.00 per cent after 10th days treatment respectively.

At depth of more than 20.00 cms *i.e.* 25.00, 30.00 and 35.00 cms no mortality 0.00 per cent was recorded.

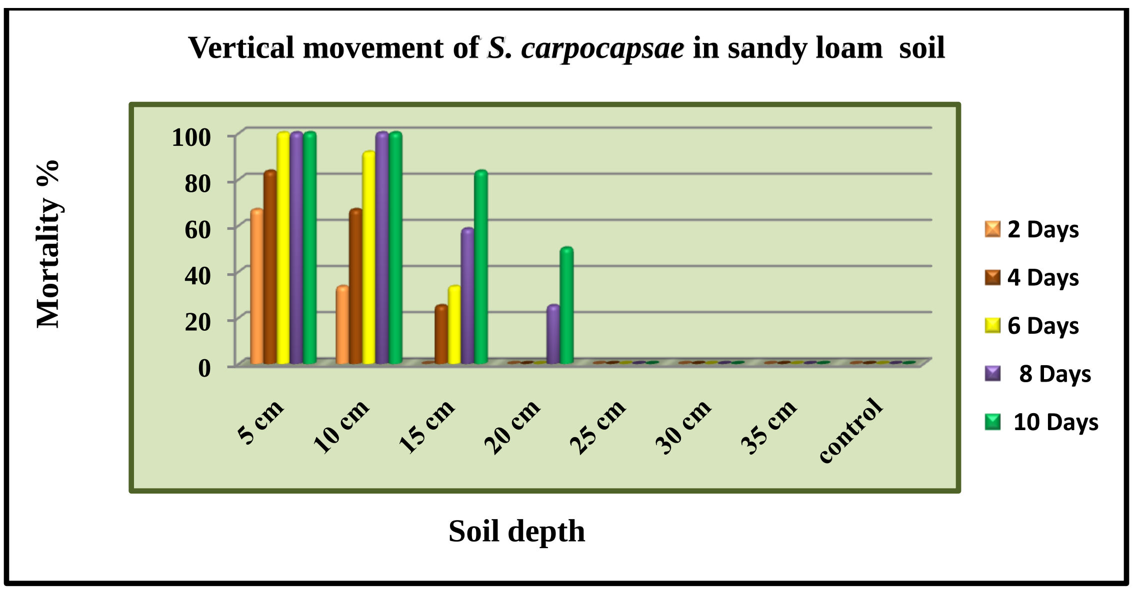



**Fig.4.19 Vertical movement of *S. carpocapsae* in sandy loam soil**

### 4.2.4.3 Effect of soil depth on vertical movement of *S. carpocapsae* in clay soil

Studies conducted to record the vertical movement of *S. carpocapsae* at different depths and duration of time in clay soil as indicated by the mortality of the larvae of *G. mellonella* was analysed in CRD (Completely randomized design) replicated three times from the data, it is clear that as the depth of the soil increased, resulted in decreased mortality of the test insects, *G. mellonella* larvae in the species of EPNs tested.

**Table No. 4.10 Vertical movement of *S. carpocapsae* at different depths (cms) and duration of time (days) in clay soil**

| Treatment | Percent mortality of *G. mellonella* larvae in clay soil at different time interval (days) | | | | | |
|---|---|---|---|---|---|---|
| | 2 days | 4 days | 6 days | 8 days | 10 days | Mean |
| **5.00 cm** | 0.00 (0.06) | 8.33 (9.99) | 16.66 (19.99) | 58.33 (49.98) | 83.33 (69.98) | 33.33 |
| **10.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 33.33 (34.98) | 50.00 (44.98) | 16.66 |
| **15.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 8.33 (9.99) | 1.66 |
| **20.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **25.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **30.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **35.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **Control** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **SE(m)** | - | 3.53 | 3.53 | 2.49 | 5.86 | - |
| **C.D.(1%)** | - | NS | 10.68 | 7.55 | 17.72 | - |

****Figures in parentheses are arc sine transformed value**

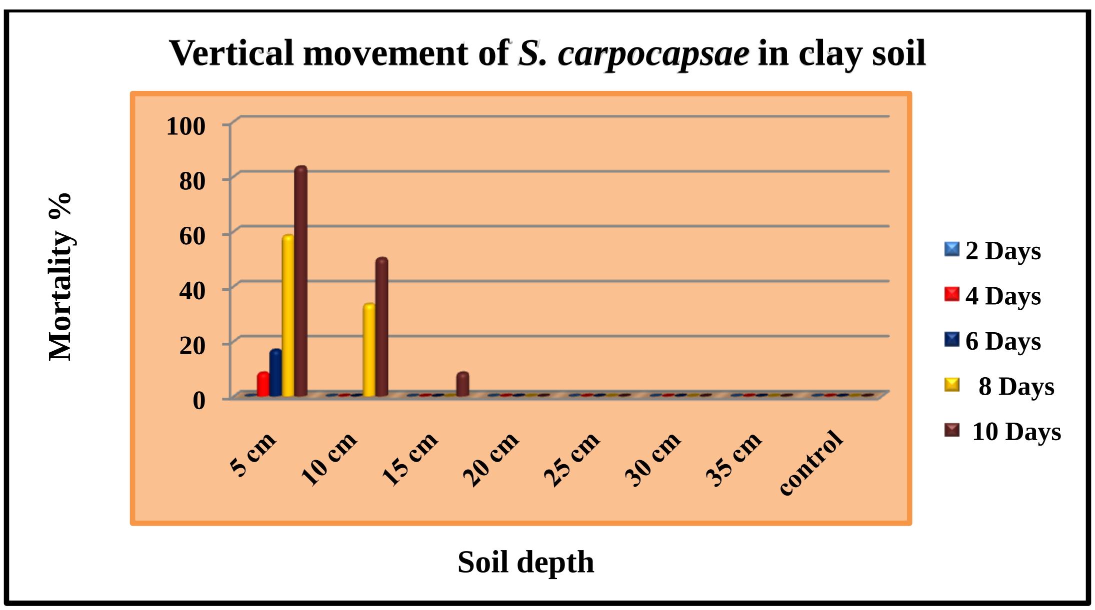



**Fig.4.20 Vertical movement of *S. carpocapsae* in clay soil**

Maximum mortality of the larvae was observed at the depth of 5.00 cms. Observations recorded after two days of inoculation of 1000 IJs, no mortality was recorded on 2$^{nd}$ day which increased to 8.33, 16.66, 58.33 and 83.33 per cent after 4$^{th}$, 6$^{th,}$ 8$^{th}$ and 10$^{th}$ days after treatment respectively.

At 10.00 cms depth no mortality was recorded on the 2$^{nd}$, 4$^{th}$ and 6$^{th}$ days from 8$^{th}$ 33.33 per cent mortality was observed, which increased to 50.00 per cent after 10$^{th}$ days treatment.

At 15.00 cms depth no mortality was recorded on the 2$^{nd}$, 4$^{th}$, 6$^{th}$ and 8$^{th}$ day and from 10$^{th}$ days 8.33 per cent mortality was observed. At depth of more than 15.00 cms *i.e.* 20.00, 25.00, 30.00 and 35.00 cms no mortality 0.00 per cent was recorded.

**4.2.4.4 Effect of soil depth on vertical movement of *H. indica* in sandy soil**

Studies conducted to record the vertical movement of *H. indica* at different depths in sandy soil at duration of time as indicated by the mortality of the larvae of *G. mellonella* was analysed in CRD (Completely randomized design) replicated three times from the data, it is clear that as the depth of the soil increased, resulted in decreased mortality of the test insects, *G. mellonella* larvae in the species of EPNs tested.

Maximum mortality of the larvae was observed at the depth of 5.00 cms. Observations recorded two days after inoculation of 1000 IJs, depicted 66.66 per cent mortality on 2$^{nd}$ day which increased to 91.66 and 100.00 per cent after 4$^{th}$ and 6$^{th}$ days after treatment respectively.

At 10.00 cms depth no mortality was recorded on the 2$^{nd}$ day and from 4$^{th}$ day mortality of 41.66 per cent was observed, which increased to 91.66 and 100.00 per cent after 6$^{th}$ and 8$^{th}$ days after treatment respectively.

At 15.00 cms depth no mortality was recorded on the 2$^{nd}$ and 4$^{th}$ days after treatment and mortality was observed from 6$^{th}$, 8$^{th}$ and 10$^{th}$ days as 41.66, 66.66 and 83.33 per cent respectively.

At depth of more than 20.00 cms *i.e.* 20.00, 25.00, 30.00 and 35.00 cms no mortality 0.00 per cent was recorded.

**Table No. 4.11 Vertical movement of *H. indica* at different depths (cms) and duration of time (days) in sandy soil**

| Treatment | Percent mortality of *G. mellonella* larvae in sandy soil at different time interval (days) | | | | | |
|---|---|---|---|---|---|---|
| | **2 days** | **4 days** | **6 days** | **8 days** | **10 days** | **Mean** |
| **5.00 cm** | 66.66 (54.97) | 91.66 (79.99) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 91.66 |
| **10.00 cm** | 0.00 (0.06) | 41.66 (39.98) | 91.66 (79.99) | 100.00 (90.00) | 100.00 (90.00) | 66.66 |
| **15.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 41.66 (39.98) | 66.66 (54.97) | 83.33 (69.98) | 38.33 |
| **20.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 33.33 (34.98) | 6.66 |
| **25.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **30.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **35.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **Control** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **SE(m)** | 1.76 | 3.95 | 3.95 | 1.76 | 3.95 | - |
| **C.D.(1%)** | 5.34 | 11.95 | 11.95 | 5.34 | 11.95 | - |

****Figures in parentheses are arc sine transformed value**

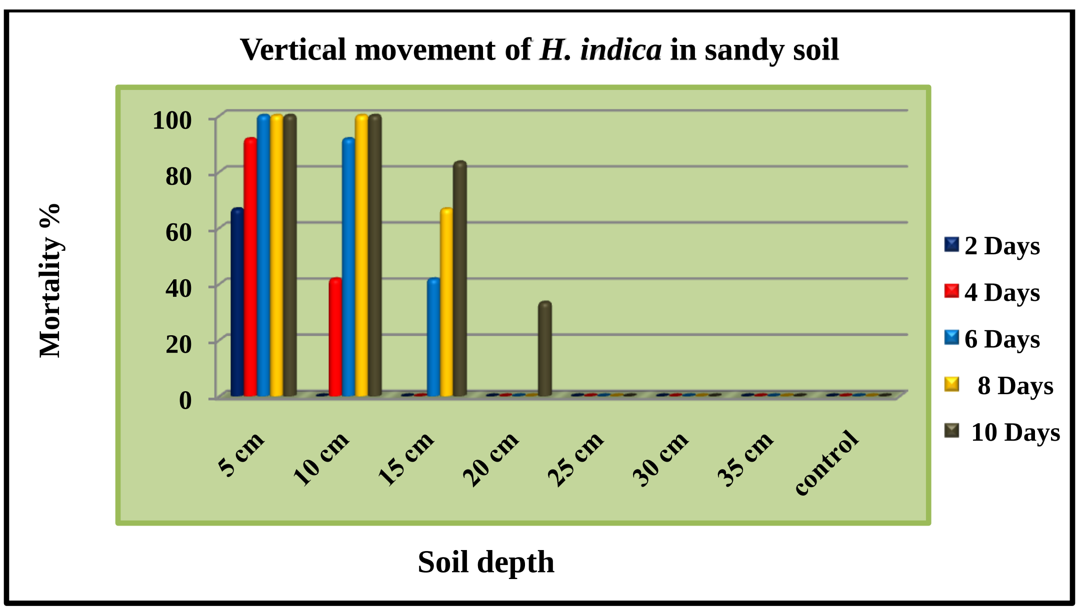



**Fig.4.21 Vertical movement of *H. indica* in sandy soil.**

Studies conducted to record the vertical movement of *H. indica* at different depths in sandy soil at duration of time as indicated by the mortality of the larvae of *G. mellonella* was analysed in CRD (Completely randomized design) replicated three times from the data, it is clear that as the depth of the soil increased, resulted in decreased mortality of the test insects, *G. mellonella* larvae in the species of EPNs tested.

Maximum mortality of the larvae was observed at the depth of 5.00 cms. Observations recorded two days after inoculation of 1000 IJs, depicted 33.33 per cent mortality on 2$^{nd}$ day which increased to 75.00, 83.33 and 100.00 per cent after 4, 6 and 8 days after treatment respectively.

At 10.00 cms depth no mortality was recorded on the 2$^{nd}$ day and from 4$^{th}$ day mortality of 33.33 per cent was observed, which increased to 41.66, 66.66 and 91.66 per cent after 6, 8 and 10 days after treatment.

At 15.00 cms depth no mortality was recorded on the 2$^{nd}$ 4$^{th}$ and 6$^{th}$ days after treatment and mortality observed was from 8$^{th}$ and 10$^{th}$ days as 16.66 and 33.33 per cent respectively.

At 20.00 cms depth no mortality was recorded on the 2$^{nd}$, 4$^{th}$, 6$^{th}$ and 8$^{th}$ day and from 10$^{th}$ days 33.33 per cent mortality was observed.

At depth of more than 20.00 cms *i.e.* 25.00, 30.00 and 35.00 cms no mortality 0.00 per cent was recorded.

**4.2.4.5 Effect of soil depth on vertical movement of *H. indica* in sandy loam soil**

Studies conducted to record the vertical movement of *H. indica* at different depths in sandy loam soil at duration of time as indicated by the mortality of the larvae of *G. mellonella* was analysed in CRD (Completely randomized design) replicated three times from the data, it is clear that as the depth of the soil increased, resulted in decreased mortality of the test insects, *G. mellonella* larvae in the species of EPNs tested.

Maximum mortality of the larvae was observed at the depth of 5.00 cms. Observations recorded after two days of inoculation of 1000 IJs, where 91.66 per cent mortality was recorded on 2$^{nd}$ day which increased to 100.00 per cent after 4 days after treatment respectively.

**Table No. 4.12. Vertical movement of *H. indica* at different depths (cms) and duration of time (days) in sandy loam soil**

| Treatment | Percent mortality of *G. mellonella* larvae in sandy loam soil at different time interval (days) | | | | | |
|---|---|---|---|---|---|---|
| | **2 days** | **4 days** | **6 days** | **8 days** | **10 days** | **Mean** |
| **5.00 cm** | 91.66 (79.99) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 98.33 |
| **10.00 cm** | 58.33 (49.98) | 83.33 (69.98) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 88.33 |
| **15.00 cm** | 25.00 (29.98) | 33.33 (34.98) | 58.33 (49.98) | 100.00 (90.00) | 100.00 (90.00) | 63.33 |
| **20.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 41.66 (39.98) | 75.00 (64.98) | 83.33 (69.98) | 39.99 |
| **25.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 8.33 (9.99) | 1.66 |
| **30.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **35.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **Control** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **SE(m)** | 3.95 | 3.95 | 2.49 | 4.67 | 3.53 | - |
| **C.D.(1%)** | 11.95 | 11.95 | 7.55 | 14.14 | 10.69 | - |

****Figures in parentheses are arc sine transformed value**

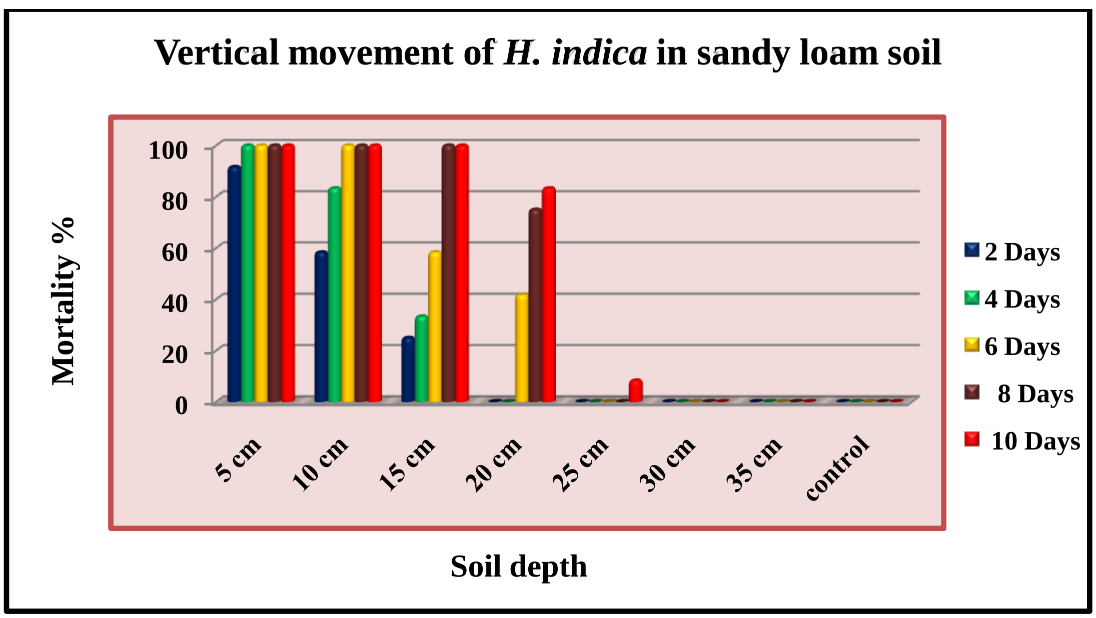



**Fig.4.22 Vertical movement of *H. indica* in sandy loam soil**

At 10.00 cms depth 58.33 per cent mortality was recorded on the 2nd day which increased to 83.33 and 100.00 per cent after 4th and 6th days after treatment respectively.

At 15.00 cms depth 25.00 per cent mortality was observed on the 2nd day, which increased to 33.33, 58.33, 83.33 and 100.00 per cent after 4th, 6th and 8th day treatment respectively.

At 20.00 cms depth no mortality was recorded on the 2nd and 4th days and from 6th day mortality of 41.66 per cent was observed, which increased to 75.00 and 83.33 per cent after 8th and 10th days treatment respectively.

At 25.00 cms depth no mortality was recorded on the 2nd, 4th, 6th and 8th day and from 10th days 8.33 per cent mortality was observed.

At depth of more than 25.00 cms *i.e.* 30.00 and 35.00 cms no mortality 0.00 per cent was recorded.

**4.2.4.6 Effect of soil depth on vertical movement of *H. indica* in clay soil**

Studies conducted to record the vertical movement of *H. indica* at different depths in sandy loam soil at duration of time as indicated by the mortality of the larvae of *G. mellonella* was analysed in CRD (Completely randomized design) replicated three times from the data, it is clear that as the depth of the soil increased, resulted in decreased mortality of the test insects, *G. mellonella* larvae in the species of EPNs tested.

Maximum mortality of the larvae was observed at the depth of 5.00 cms. Observations recorded after two days of inoculation of 1000 IJs, no mortality was recorded on 2nd day which increased to 16.66, 41.66, 83.33 and 100.00 per cent after 4th, 6th, 8th and 10th days after treatment respectively.

At 10.00 cms depth no mortality was recorded on the 2nd and 4th days from 6th 33.33 per cent mortality was observed, which increased to 58.33 and 75.00 per cent after 8th and 10th days treatment.

At 15.00 cms depth no mortality was recorded on the 2nd, 4th, 6th and 8th day and from 10th days 16.66 per cent mortality was observed. At depth of more than 15.00 cms *i.e.* 20.00, 25.00, 30.00 and 35.00 cms no mortality 0.00 per cent was recorded.

**Table No. 4.13 Vertical movement of *H. indica* at different depths (cms) and duration of time (days) in clay soil**

| Treatment | Percent mortality of *G. mellonella* larvae in clay soil at different time interval (days) | | | | | |
|---|---|---|---|---|---|---|
| | **2 days** | **4 days** | **6 days** | **8 days** | **10 days** | **Mean** |
| **5.00 cm** | 0.00 (0.06) | 16.66 (19.99) | 41.66 (39.98) | 83.33 (69.98) | 100.00 (90.00) | 48.00 |
| **10.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 33.33 (34.98) | 58.33 (49.98) | 75.00 (64.98) | 33.33 |
| **15.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 16.66 (19.99) | 3.33 |
| **20.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **25.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **30.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **35.00 cm** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **Control** | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 (0.06) | 0.00 |
| **SE(m)** | - | 3.53 | 2.49 | 3.95 | 4.67 | - |
| **C.D.(1%)** | - | 10.68 | 7.55 | 11.95 | 14.14 | - |

****Figures in parentheses are arc sine transformed value**

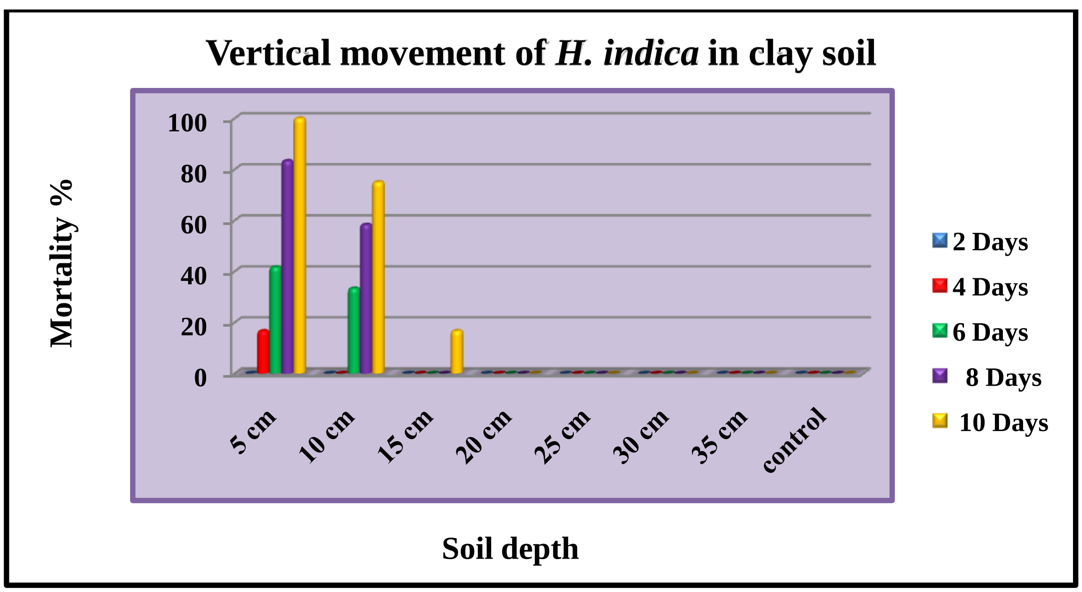



**Fig.4.23 Vertical movement of *H. indica* in clay soil**

**Table No.4.14 Mean vertical movement of EPNs at different depth and types of soil.**

| Treatment | Sandy soil | Sandy loam soil | Clay soil | Sandy soil | Sandy loam soil | Clay soil |
|---|---|---|---|---|---|---|
| | | ***S. carpocapsae*** | | | ***H. indica*** | |
| **5.00 cm** | 78.33 | 89.99 | 33.33 | 91.66 | 98.33 | 48.00 |
| **10.00 cm** | 46.66 | 78.33 | 16.66 | 66.66 | 88.33 | 33.33 |
| **15.00 cm** | 9.99 | 39.99 | 1.66 | 38.33 | 63.33 | 3.33 |
| **20.00 cm** | - | 15.00 | - | 6.66 | 39.99 | - |
| **25.00 cm** | - | - | - | - | 1.66 | - |
| **30.00 cm** | - | - | - | - | - | - |
| **35.00 cm** | - | - | - | - | - | - |
| **Control** | - | - | - | - | - | - |
| **SE(m)** | - | - | - | - | - | - |
| **C.D.** | - | - | - | - | - | - |

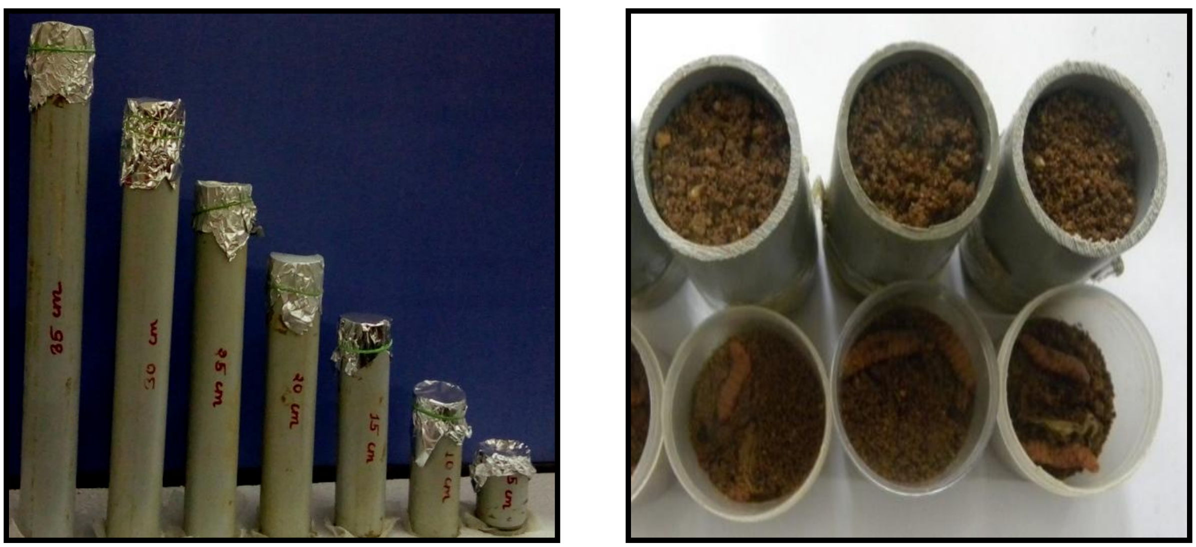



**Plate No. 16 Mortality of *G. mellonella* at different depth**

The data revealed that the inoculation of 1,000 IJs of *S. carpocapsae* in different types of soil with different depth, highest mortality of *G. mellonella* larvae was recorded of 89.99 to 15.00 per cent at 5.00 to 20.00 cm in sandy loam soil followed by 78.33 to 9.99 per cent at 5.00 to 15.00 cm in sandy soil, and lowest mortality recorded of 33.33 to 1.66 per cent at 5.00 to 15.00 cm in clay soil.

Similarly, in case of *H. indica* the highest mortality of *G. mellonella* larvae was recorded of 98.33 to 1.66 per cent at 5.00 to 25.00 cm in sandy loam soil followed by 91.66 to 6.66 per cent at 5.00 to 15.00 cm in sandy soil, and lowest mortality recorded of 48.00 to 3.33 per cent at 5.00 to 15.00 cm in clay soil. Hence, from the above results it can be concluded that there was *H.indica* have more host searching ability than *S. carpocapsae*.

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. Barbercheck and Kaya (1991a) also have similar findings 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.

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 vertical movement decreased. 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.

## 4.3 To study the mass multiplication of EPNs at laboratory condition on different insects hosts

### 4.3.1 Mass multiplication of EPNs on larvae of *G. mellonella* of different length and weight

Data pertaining to mass multiplication of both the species of EPNs *i.e. S. carpocapsae* and *H. indica* on different length (in mm) and body weight (in mg) of larvae of *G. mellonella* as a test insect were recorded and presented in Table No. 4.15. The data on the yield of IJs revealed that the production of IJs per larva was directly proportional to the length and body weight of the tested insect. The IJs of both the EPNs species *i.e. S. carpocapsae* and *H. indica* were successfully cultured on all the three sizes of test insects. However, there were significant differences on the number of IJs produced on different sizes of the larvae.

For mass multiplication of IJs of *S. carpocapsae* on *G. mellonella* larva of different length and body weight were taken. The length and body weight of *G. mellonella* ranged from 9-11mm and 140-145 mg in small category, 15–16 mm and 210-220 mg in medium category and 20–21mm and 300-315 mg in large category were tested. The data on the yield of IJs of *S. carpocapsae* revealed that the production of IJs per larva was directly proportional to the length and body weight of the insect species tested. An average number of 84,317.5 IJs of *S. carpocapsae* were produced in small, 91,513.5 in medium and 1,07,104 in large sized larvae of *G. mellonella.* The mean minimum and mean maximum number of IJs of *S. carpocapsae* produced ranged from 79,679 to 88,956 IJs, 85,460 to 97,567 IJs and 1,01,245 to 1,12,963 IJs in small, medium and large sized larva of *G. mellonella* respectively. A pooled mean production of IJs of *S. carpocapsae* was computed to an yield of 94,311.66 IJs from small, medium and large sized host larvae of *G. mellonella,* was recorded (Table). However, the IJs of *S. carpocapsae* were successfully cultured on all the three sizes of test insects.

The data on the yield of IJs of *H. indica* also revealed that the production of IJs per larva was directly proportional to the size and body weight of the insect species tested. The average number of IJs of *H. indica* was 1,06,560.5 in small, 1, ,59,258.5 in medium and 2,38,885.5 in large sized larva of *G. mellonella*. The mean minimum and mean maximum number of IJs of *H. indica* produced ranged from 1,03,495 to 1,09,626 IJs, 1,56,374 to 1,62,143 IJs and 2,35,132 to 2,42,639 IJs in small, medium and large sized larva of *G. mellonella* respectively. While computing the pooled mean production of IJs of *H. indica* a yield of 1,68,234.83 IJs host larval of *G. mellonella*, was recorded (Table ). The IJs of *H. indica* were successfully cultured on all three sizes of the test insect.

Looking to the progeny production, *S. carpocapsae* produced an average of 94,311.66 IJs while that of *H. indica* produced 1,68,234.83 IJs per larva of *G. mellonella*.

However, there was a considerable difference in number of IJs production among the two EPNs *i.e. S.carpocapsae* and *H.indca,* which might be due to large size of the larva able to provide sufficient nourishment for the developing infective juveniles as compared to small sized larvae. These results are in line with results of Rajkumar *et. al.* (2002) who also obtained high progeny yield of *H. indica* from large sized larva (length of 20 to 22 mm, weight of 223 to 257 mg) *S. carpocapsae* than as revealed in the present studies.

**Table No. 4.15 Mass multiplication of EPNs from different sized larvae of *G. mellonella***

| **Insect species** | **EPNs species** | **Average length of insect larvae (mm)** | **Average weight of larvae (mg)** | **No. of IJs produced /mg body weight of larva** | | | |
|---|---|---|---|---|---|---|---|
| | | | | **Min.** | **Max.** | **Average** | **Pooled mean** |
| ***G. mellonella*** | ***S. carpocapsae*** | Small (9–11) | 140-145 | 79,679 | 88,956 | 84,317.5 | 94,311.66 |
| | | Medium (15–16) | 210-220 | 85,460 | 97,567 | 91,513.5 | |
| | | Large (20–21) | 300-315 | 1,01,245 | 1,12,963 | 1,07,104 | |
| | ***H. indica*** | Small (9–11) | 140-145 | 1,03,495 | 1,09,626 | 1,06,560.5 | 1,68,234.83 |
| | | Medium (15–16) | 210-220 | 1,56,374 | 1,62,143 | 1,59,258.5 | |
| | | Large (20–21) | 300-315 | 2,35,132 | 2,42,639 | 2,38,885.5 | |

**Average of 10 larvae**

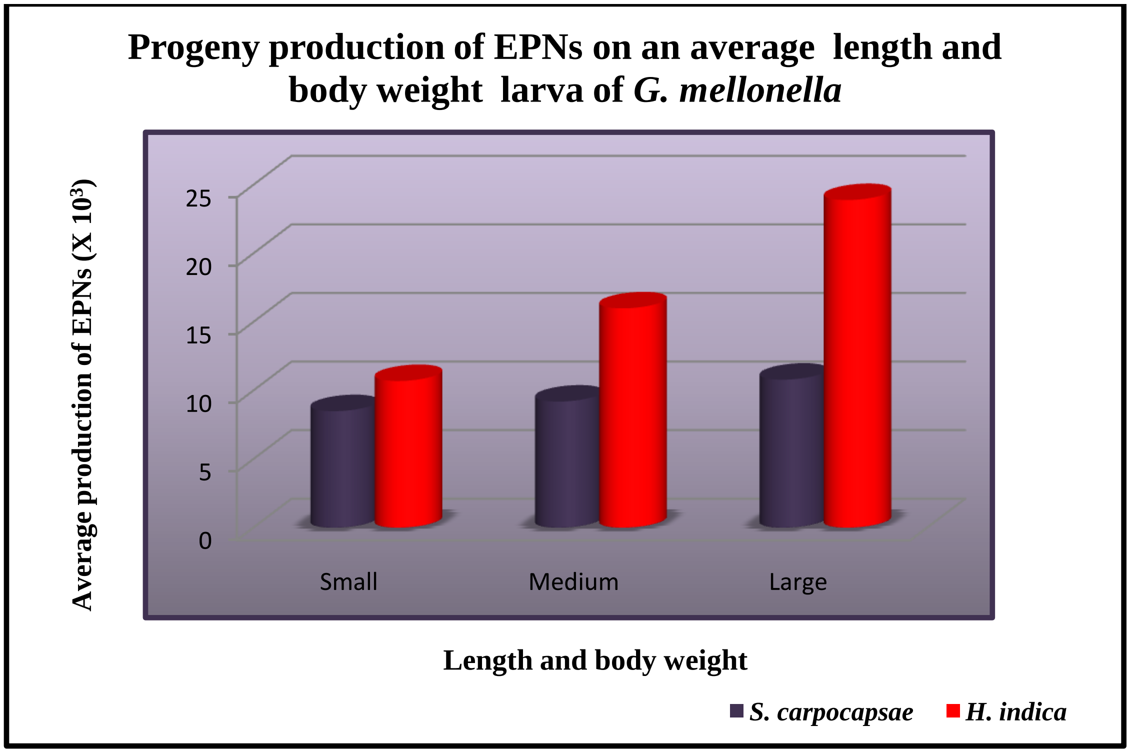



**Fig.4.24** **Progeny production of EPNs on an average length and body weight larva of *G. mellonella.***

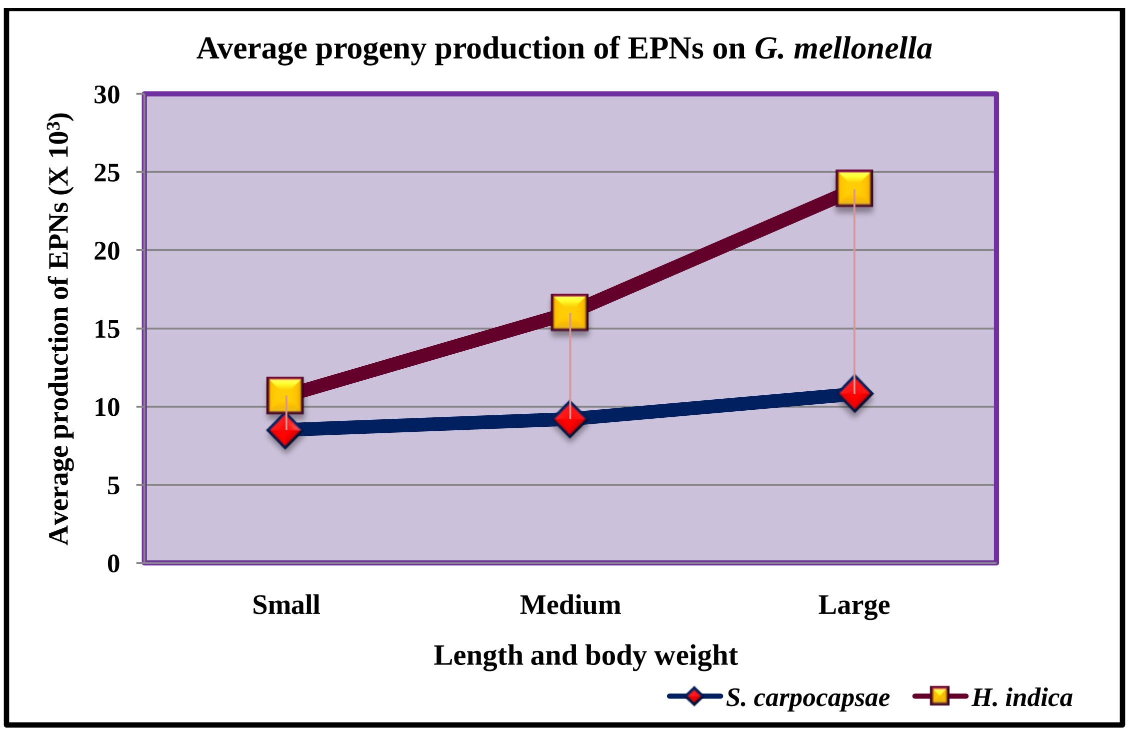



**Fig.4.25 Effect of different length and weight of *G. mellonella* on progeny production of EPNs.**

### 4.3.2 Mass multiplication of EPNs on larvae of *C. cephalonica* of different length and weight.

Data pertaining to mass multiplication of both the species of EPNs *via. S. carpocapsae* and *H. indica* on different sized *i.e.* small, medium and large (length in mm and body weight in mg) of larvae of *C. cephalonica* as a test insect were recorded and presented in Table No. 4.16. The data on the yield of IJs revealed that the production of IJs per larva was directly proportional to the size and body weight of the tested insect. The IJs of both the EPNs were successfully cultured on all the three sizes of test insects.

For mass multiplication of IJs of *S. carpocapsae* on *C. cephalonica,* larva of different length and body weight were taken. The length and body weight of *C. cephalonica* ranged from 5 - 7 mm and 40-45 mg in small category, 9–11 mm and 50-55 mg in medium category and 15-17 mm and 70-80 mg in large category, were tested. The data on the yield of IJs of *S. carpocapsae* revealed that the production of IJs per larva was directly proportional to the length and body weight of the insect species tested. An average number of 68,960.5 IJs of *S. carpocapsae* were produced in small, 83,435.5 in medium and 1,01,058.5 in large sized larvae of *C. cephalonica*. The mean minimum and mean maximum number of IJs of *S. carpocapsae* produced ranged from 64,329 to 73,592 IJs, 78,636 to 88,235 IJs and 93,526 to 1,08,591 IJs in small, medium and large sized larva of *C. cephalonica* respectively. The pooled mean production of IJs of *S. carpocapsae* was computed to a yield of 84,484.83 IJs from small, medium and large sized larvae of *C. cephalonica,* was recorded (Table ). However, the IJs of *S. carpocapsae* were successfully cultured in all the three sizes of test insects.

Thus, on an average *H. indica* produced 90,974 in small, 1,06,857 in medium and 1,29,453.5 number of IJs in large sized of larva of *C. cephalonica*. The mean minimum and mean maximum number of *H. indica* produced IJs ranged from 86,521 to 95,427 IJs, 1,01,361 to 1,12,353 and 1,22,325 to 1,36,582 IJs in small, medium and large sized larva of *C. cephalonica* respectively. The pooled mean production of IJs of *H. indica* was computed to an yield of 1,09,094.83 IJs from small, medium and large sized host larvae of *C. cephalonica*. However, the IJs of *H. indica* were successfully cultured in all the three sizes of insect tested.

**Table No. 4.16 Mass multiplication of EPNs from different sized larvae of *C. cephalonica***

| Insect species | EPNs species | Average length of insect larvae (mm) | Average weight of larva (mg) | No. of IJs produced / mg body weight of larva | | | |
|---|---|---|---|---|---|---|---|
| | | | | **Min.** | **Max.** | **Average** | **Pooled mean** |
| ***C. cephalonica*** | ***S. carpocapsae*** | Small (5 - 7) | 40-45 | 64,329 | 73,592 | 68,960.5 | 84,484.83 |
| | | Medium (9 - 11) | 50-55 | 78,636 | 88,235 | 83,435.5 | |
| | | Large (15 - 17) | 70-80 | 93,526 | 1,08,591 | 1,01,058.5 | |
| | ***H. indica*** | Small (5 - 7) | 40-45 | 86,521 | 95,427 | 90,974 | 1,09,094.83 |
| | | Medium (9 - 11) | 50-55 | 1,01,361 | 1,12,353 | 1,06,857 | |
| | | Large (15 - 17) | 70-80 | 1,22,325 | 1,36,582 | 1,29,453.5 | |

**Average of 10 larvae**

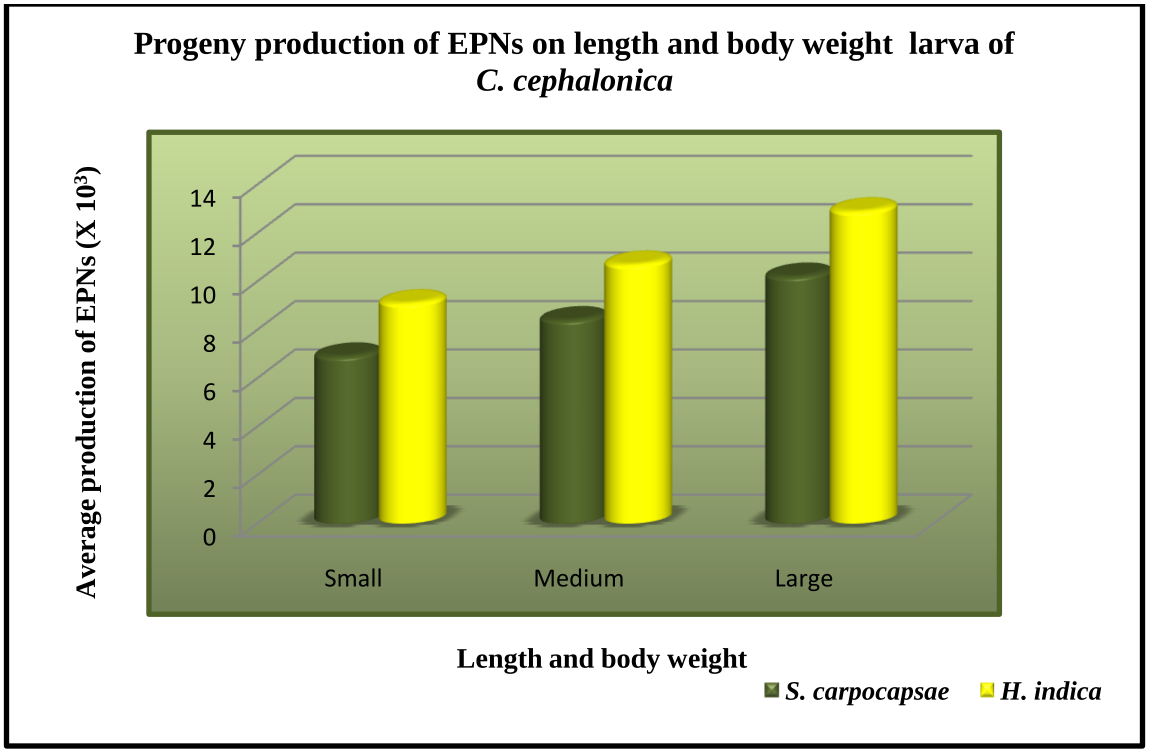



**Fig.4.26** **Progeny production of EPNs on an average size and body weight larva of *C. cephalonica***

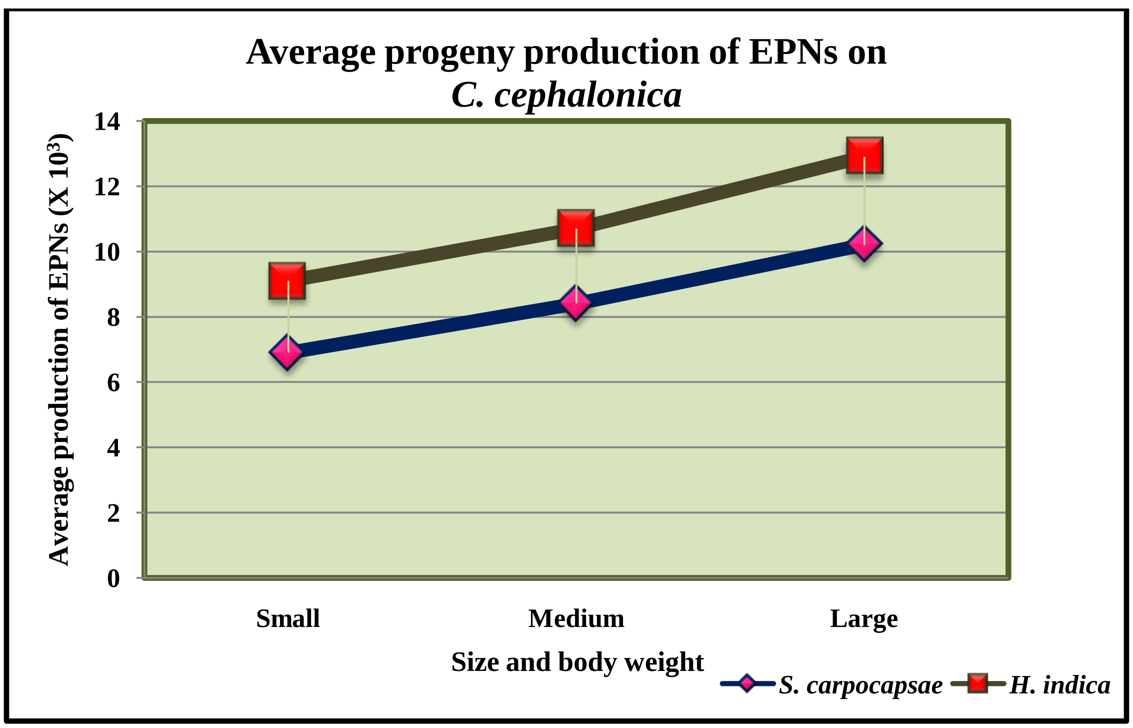



**Fig.4.27 Effect of different length and weight of *C. cephalonica* on progeny production of EPNs**

### 4.3.3 Mass multiplication of entomopathogenic nematodes on fifth instar larvae *G. mellonella*

Results pertaining to the progeny production of the *S. carpocapsae* on the fifth instar larvae of *G. mellonella* presented in the Table No. 4.17 revealed that as the number of IJs inoculum was increased, there was a simultaneous significant increase in yield of the IJs. Significantly highest progeny yield of 2,42,119.50 recorded was with an inoculum of 60 IJs/ larva followed by 80 and 100 IJs of 2,34,240.50 and 2,00,983.30 numbers of IJs/ larva respectively. At minimum dosage of 20 IJs per larva, resulted in a mean progeny production of 1,20,957.00 numbers of IJs followed by 40 IJs with a production of 1,73,546.50 numbers of IJs.

Results pertaining to the production of *H. indica* on fifth instar larvae of *G. mellonella* as presented in the Table No. 4.17 showed that *H. indica* infected larvae turned to brick red to dark brown colour. As the number of IJs inoculum increased, there was a significant increase in yield of the IJs on larva. Significantly highest progeny yield was recorded of 4,82,084.50 IJs with an inoculum of 80 IJs/ larva followed by 100 IJs with 4,08,764.80 IJs/ larva respectively. Lower dosage of 20, 40 and 60 IJs per larva, resulted in a mean progeny production of 3,24,269.50, 3,49,260.00 and 4,37,234.80 numbers of IJs/ larva respectively.

Significantly higher progeny production in the case of *H. indica* with maximum progeny of 4,82,084.50 IJs/ larva was recorded at an inoculum level of 80 IJs per larva in *G. mellonela* was obtained as compared 2,34,240.50 IJs of *S. carpocapsae* at the same dose of inoculum. This might be due to the reason that a single larva of *G. mellonella* could accommodate more number of infective juveniles of *H. indica* that yielded maximum of 4,82,084.50 IJs/ larva as compared to 2,34,240.50 IJs of *S. carpocapsae.* This may be due to the reason that a single larva of *G. mellonella* could not provide the required food material for 80 IJS of *S. carpocapsae* as compared to *H. indica*. These results are more or less similar to those reported by P.D.B.C (2002) where they obtained highest infective juveniles 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 of inoculum level.

**Table No. 4.17 Mass multiplication of EPNs with different inoculum on the fifth instar larvae *G. mellonella***

| Inoculum level (No. of IJs/ larva) | Progeny yield infective juveniles in lakhs/ larva | |
|---|---|---|
| | ***S. carpocapsae*** | ***H. indica*** |
| **20.00** | 1,20,957.00 (347.73) | 3,24,269.50 (569.43) |
| **40.00** | 1,73,546.50 (416.54) | 3,49,260.00 (590.91) |
| **60.00** | 2,42,119.50 (491.96) | 4,37,234.80 (661.22) |
| **80.00** | 2,34,240.50 (483.90) | 4,82,084.50 (694.26) |
| **100.00** | 2,00,983.30 (448.19) | 4,08,764.80 (639.30) |
| **Control** | 0.00 (0.02) | 0.00 (0.02) |
| **SE(m)** | 4.456 | 3.76 |
| **C.D.(1%)** | 13.342 | 11.26 |

****Figures in parentheses are square root transformed values**

**Plate No. 17 Progeny production of EPNs from the cadavers.**

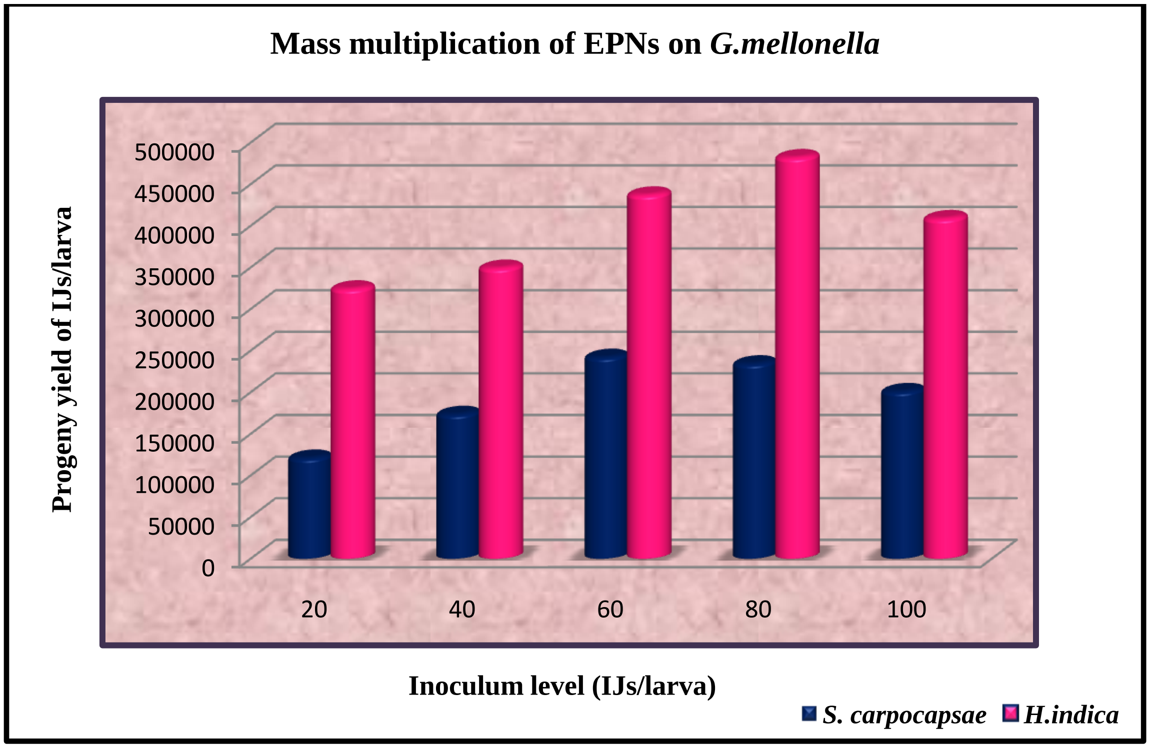



**Fig.4.28 Mass multiplication of EPNs on *G.mellonella* with different IJs inoculum**

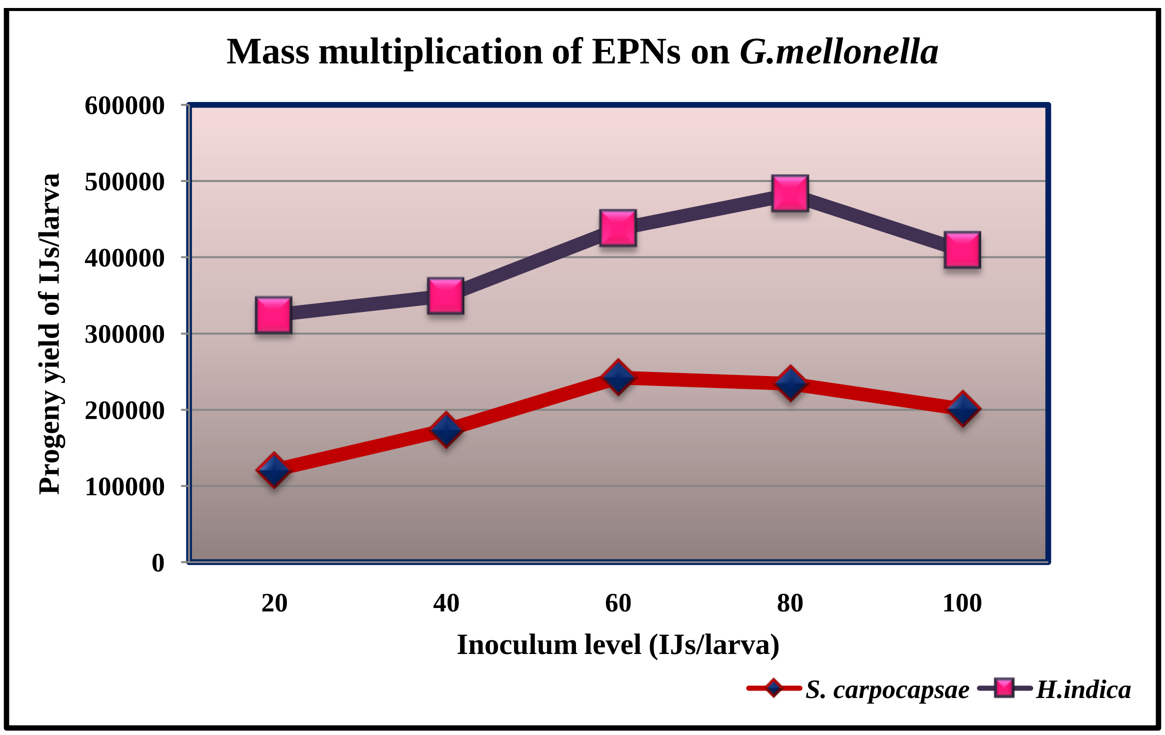



**Fig.4.29 Mass multiplication of EPNs on *G.mellonella* with different IJs inoculum**

### 4.3.4 Mass multiplication of entomopathogenic nematodes on *C. cephalonica*

Results pertaining to the mass production of *S. carpocapsae* on fifth instar larvae of *C. cephalonica* as presented in the Table No. 4.18 revealed that, as the number of IJs inoculum was increased, there was a significant increase in yield of the IJs in the larva. Significantly highest progeny yield of 1,65,388.80 IJs were recorded with an inoculum of 60 IJs/ larva followed by 80 and 100 IJs with a production of 1,51,429.00 and 1,35,668.50 numbers of IJs/ larva respectively. At the minimum dosage of 20 IJs per larva resulted in lowest mean progeny production of 88,389.50 numbers of IJs followed by 40 IJs resulting in a progeny production of 1,25,530.30 numbers of IJs.

Results pertaining to the production of *H. indica* on fifth instar larvae of *C. cephalonica,* as presented in the Table No. 4.18 showed that *H. indica* infected larvae turned to brick red to dark brown colour. As the number of IJs inoculum was increased, there was a significant increase in yield of the IJs/ larva. Highest progeny yield was recorded as 3,86,737.00 with an inoculum of 80 IJs/ larva followed by 100 IJs of 3,24,942.80 IJs/ larva respectively. Lower dosage of 20, 40 and 60 IJs per larva resulted in a mean progeny production of 2,02,735.50, 2,28,027.30 and 3,52,599.80 numbers of IJs/ larva respectively.

Higher progeny production in the case of *H. indica* with maximum progeny of 3,86,737.00 IJs/ larva was recorded at an inoculum level of 80 IJs per larva of *C. cephalonica* as compared 1,51,429.00 IJs of *S. carpocapsae* at the same dose of inoculum. This might be due to the reason that a single *C. cephalonica* larva could accommodate more number of IJs/ larva of *H. indica* as compared to *S. carpocapsae.* This may be due to the reason that a single *C. cephalonica* larva could not provide the required food material for 80 IJS of *S. carpocapsae* as compared to *H. indica*.

**Table No. 4.18 Mass multiplication of EPNs with different inoculum on *C. cephalonica***

| **Inoculum level (No. of IJs/ larva)** | **Progeny yield in number of infective juveniles/ larva** | |
|---|---|---|
| | ***S. carpocapsae*** | ***H. indica*** |
| **20.00** | 88,389.50<br>(297.08) | 2,02,735.50<br>(450.21) |
| **40.00** | 1,25,530.30<br>(354.10) | 2,28,027.30<br>(477.48) |
| **60.00** | 1,65,388.80<br>(406.64) | 3,52,599.80<br>(593.79) |
| **80.00** | 1,51,429.00<br>(389.10) | 3,86,737.00<br>(621.87) |
| **100.00** | 1,35,668.50<br>(368.28) | 3,24,942.80<br>(570.02) |
| **Control** | 0.00<br>(1.00) | 0.00<br>(1.00) |
| **SE(m)** | 4.437 | 2.561 |
| **C.D.(1%)** | 13.286 | 7.669 |

**Figures in parentheses are square root transformed values**

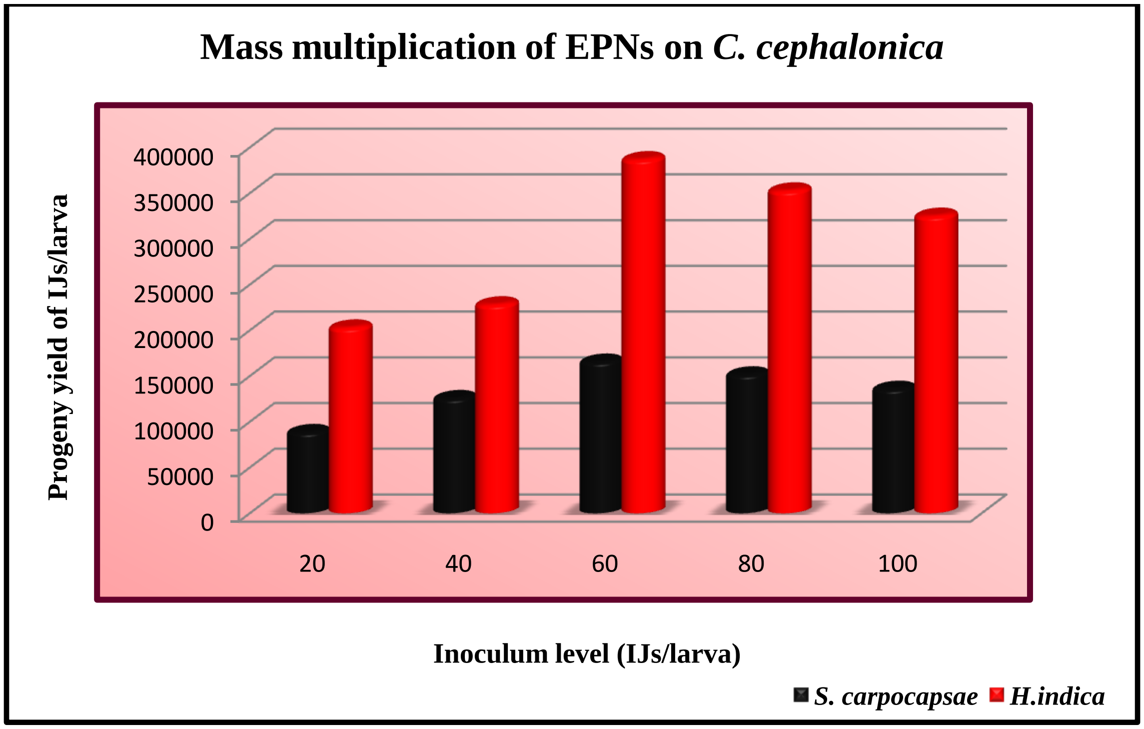



**Fig.4.30 Mass multiplication of EPNs on *C. cephalonica* with different IJs inoculum**

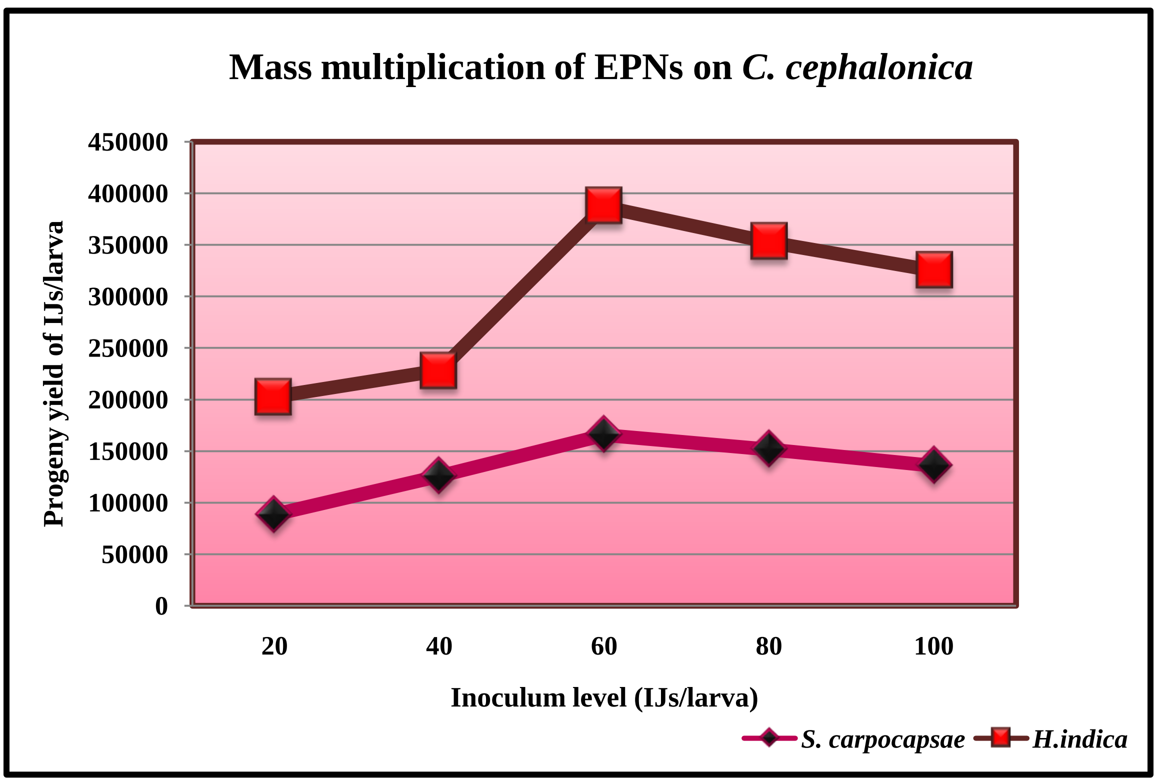



**Fig.4.31 Mass multiplication of EPNs on *C. cephalonica* with different IJs inoculum.**

### 4.3.5 Pathogenicity of EPNs on different hosts.

#### 4.3.5.1 Pathogenicity of *S. carpocapsae* on different hosts.

Experiment pertaining to the pathogenicity of the two species of EPNs *i.e. S. carpocapsae* and *H. indica* against four lepidopteran larvae *i.e. G. mellonella, C. cephalonica, Helicoverpa armigera* and *Spodoptera litura* were carried out under laboratory conditions and results obtained are documented in terms of the percentage mortality of the different larvae of the test insects and presented in Table No.4.19.

Results pertaining to the pathogenicity of *S. carpocapsae* was found to be positive in all the four insect species tested with the most preferred host as *G. mellonella* indicated by significant maximum average mortality 83.75 per cent followed by *C. cephalonica,* with 77.50 per cent, *H. armigera* and *S. litura* with 65.62 and 52.50 per cent mortality respectively within 24-48 hours. There was no mortality in control in all the four tested insects. Overall observations recorded after 24-96 hours after exposure, the treatments showed mortality range in an ascending order. As the time period increased, the mortality rate of the insect pests also increased.

In *G. mellonella*, an inoculum of 60 IJs per larva of *S. carpocapsae* of showed 52.50 per cent mortality in 24 hours of exposure. After 48 and 72 hours, mortality of 82.50 and 100.00 per cent respectively was recorded.

In *C. cephalonica,* an inoculum of 60 IJs per larva of *S. carpocapsae* showed 47.50 per cent mortality in 24 hours of exposure. After 48, 72 and 96 hours, mortality of 73.75, 88.75 and 100.00 per cent respectively was recorded.

In *H. armigera,* an inoculum of 60 IJs per larva of *S. carpocapsae* showed 45.00 per cent mortality in 24 hours of exposure. After 48, 72 and 96 hours, mortality of 62.50, 73.75 and 81.25 per cent respectively was recorded.

In *S. litura,* an inoculum of 60 IJs per larva of *S. carpocapsae* showed 36.25 per cent mortality in 24 hours of exposure. After 48, 72 and 96 hours, mortality of 47.50, 58.75 and 67.50 per cent respectively were recorded.

Thus, looking to the overall mean, the data clearly exhibits that maximum mean mortality was recorded in the larvae of *G. mellonella* of 83.75 per cent followed by *C. cephalonica* 77.50 per cent *H. armigera* 65.62 per cent and least mean mortality was observed in *S. litura* of 52.50 per cent.

**4.3.5.2 Pathogenicity of *H. indica* on different hosts.**

Results pertaining to the pathogenicity of *H. indica* was found to be positive in all the four insect species tested with most preferred host as *G. mellonella* indicated by maximum average mortality of 83.42 per cent followed by *C. cephalonica*, with 71.25 per cent, after that *H. armigera* and *S. litura* with 59.37 and 52.18 per cent mortality respectively within 24-48 hours. There was no mortality in control in all the four tested insect species. Overall observations recorded after 24-96 hours after exposure, the treatments showed mortality in an ascending order. As the time period increased, the mortality of the insect pests also increased.

In *G. mellonella*, an inoculum of 60 IJs per larva of *H. indica* showed 47.50 per cent mortality in 24 hours of exposure. After 48 and 72 hours, 86.25, 100.00 per cent respectively.

In *C. cephalonica*, an inoculum of 60 IJs per larva of *H. indica* showed 40.00 per cent mortality in 24 hours of exposure. After 48, 72 and 96 hours, there was 67.50, 77.50 and 100.00 per cent respectively.

In *H. armigera*, an inoculum of 60 IJs per larva of *H. indica* showed 37.50 per cent mortality in 24 hours of exposure. After 48, 72 and 96 hours, there was 57.50, 70.00 and 72.50 per cent respectively.

In *S. litura,* an inoculum of *H. indica* of 60 IJs per larva showed 33.75 per cent mortality in 24 hours of exposure. After 48, 72 and 96 hours, there was 48.75, 58.75 and 67.50 per cent mortality respectively.

Thus, looking to the overall mean, the data clearly exhibits that significantly maximum mean mortality was recorded in the larvae of *G. mellonella* of 83.43 per cent followed by *C. cephalonica* 71.25 per cent *H. armigera* 59.37 per cent and least mean mortality was observed in *S. litura* of 52.18 per cent.

Comparison of the data on pathogenicity of IJs of *S. carpocapsae* and *H. indica* on four different species of lepidopteran larvae *viz. G. mellonella*, *C. cephalonica*, *H. armigera* and *S. litura* indicated that overall highest mean mortality was recorded in *G. mellonella* of 83.75 and 83.43 by *S. carpocapsae* and *H. indica* respectively, and least mean minimum mortality was noted in *S. litura* of 52.50 and 52.18 per cent respectively which varied significantly with each other.

**Table No. 4.19 Pathogenicity of *S. carpocapsae and H. indica* on different hosts.**

| S.No. | Host insect | Inoculum level IJs/larva | % Mortality of larvae after hours | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **24** | **48** | **72** | **96** | **Mean** | **24** | **48** | **72** | **96** | **Mean** |
| | | | *S. carpocapsae* | | | | | *H. indica* | | | | |
| **1.** | **Greater wax moth *G. mellonella* Linnaeus** | 60 | 52.50 (46.42) | 82.50 (65.52) | 100.00 (90.00) | 100.00 (90.00) | 83.75 | 47.50 (43.54) | 86.25 (68.41) | 100.00 (90.00) | 100.00 (90.00) | 83.43 |
| **2.** | **Rice meal moth *C. cephalonica* Stainton** | 60 | 47.50 (43.54) | 73.75 (59.22) | 88.75 (70.73) | 100.00 (90.00) | 77.50 | 40.00 (39.20) | 67.50 (55.29) | 77.50 (61.83) | 100.00 (90.00) | 71.25 |
| **3.** | **Gram pod borer *H. armigera* Hubner** | 60 | 45.00 (42.09) | 62.50 (52.26) | 73.75 (59.26) | 81.25 (64.44) | 65.62 | 37.50 (37.69) | 57.50 (49.32) | 70.00 (56.80) | 72.50 (58.46) | 59.37 |
| **4.** | **Tobacco caterpillar *S. litura* Fabricius** | 60 | 36.25 (36.95) | 47.50 (43.54) | 58.75 (50.03) | 67.50 (55.29) | 52.50 | 33.75 (35.46) | 48.75 (44.26) | 58.75 (50.03) | 67.50 (55.29) | 52.18 |
| **5.** | **Control** | | 0.00 (0.02) | 0.00 (0.02) | 0.00 (0.02) | 0.00 (0.02) | 0.00 | 0.00 (0.02) | 0.00 (0.02) | 0.00 (0.02) | 0.00 (0.02) | 0.00 |
| | **SE(m)** | | 1.70 | 1.77 | 1.51 | 1.17 | - | 1.53 | 1.62 | 1.31 | 1.28 | - |
| | **C.D.(1%)** | | 5.18 | 5.41 | 4.61 | 3.58 | - | 4.68 | 4.93 | 3.99 | 3.91 | - |

****Figures in parentheses are arc sine transformed values.**

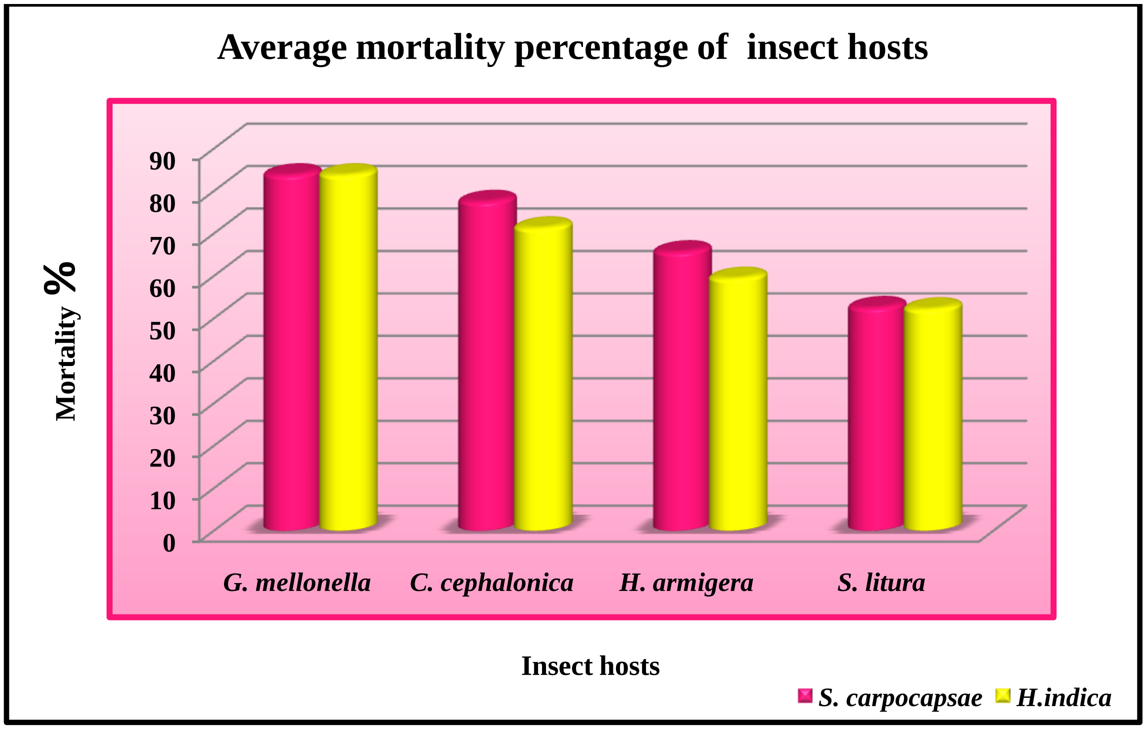



**Fig.4.32 Average mortality percentage of different insect hosts through both the EPNs**

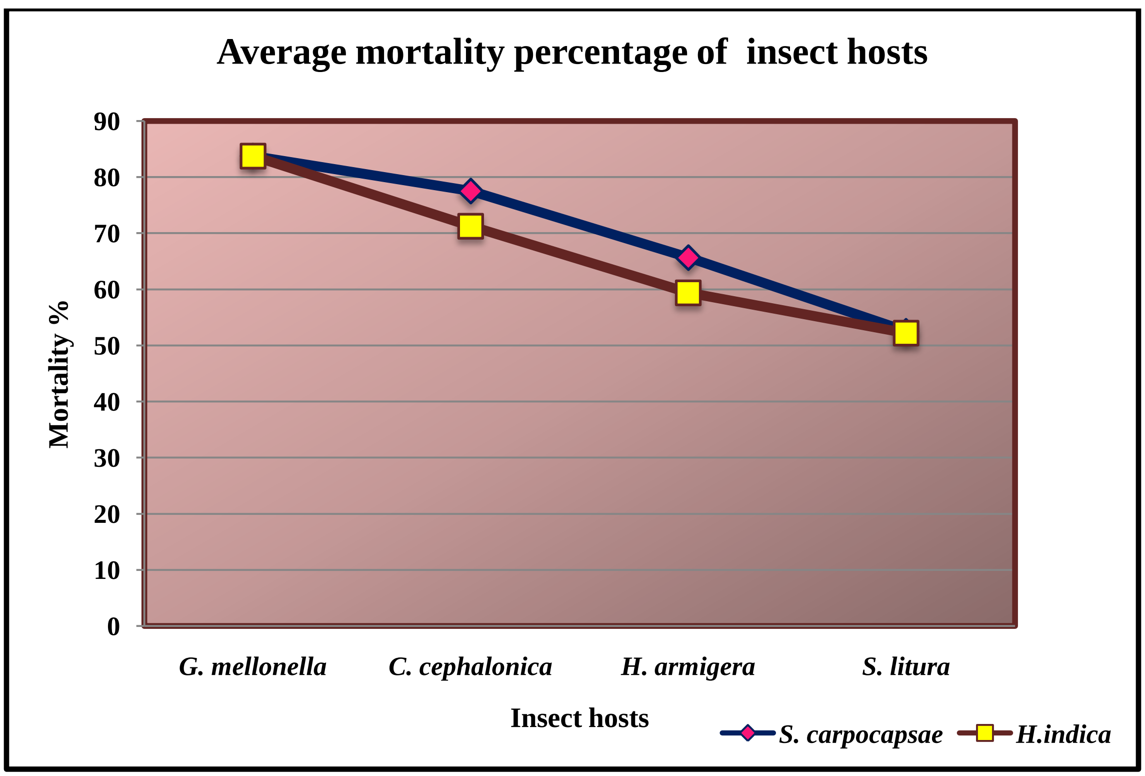



**Fig.4.33 Correlation of average mortality percentage of different insect hosts through both the EPNs**

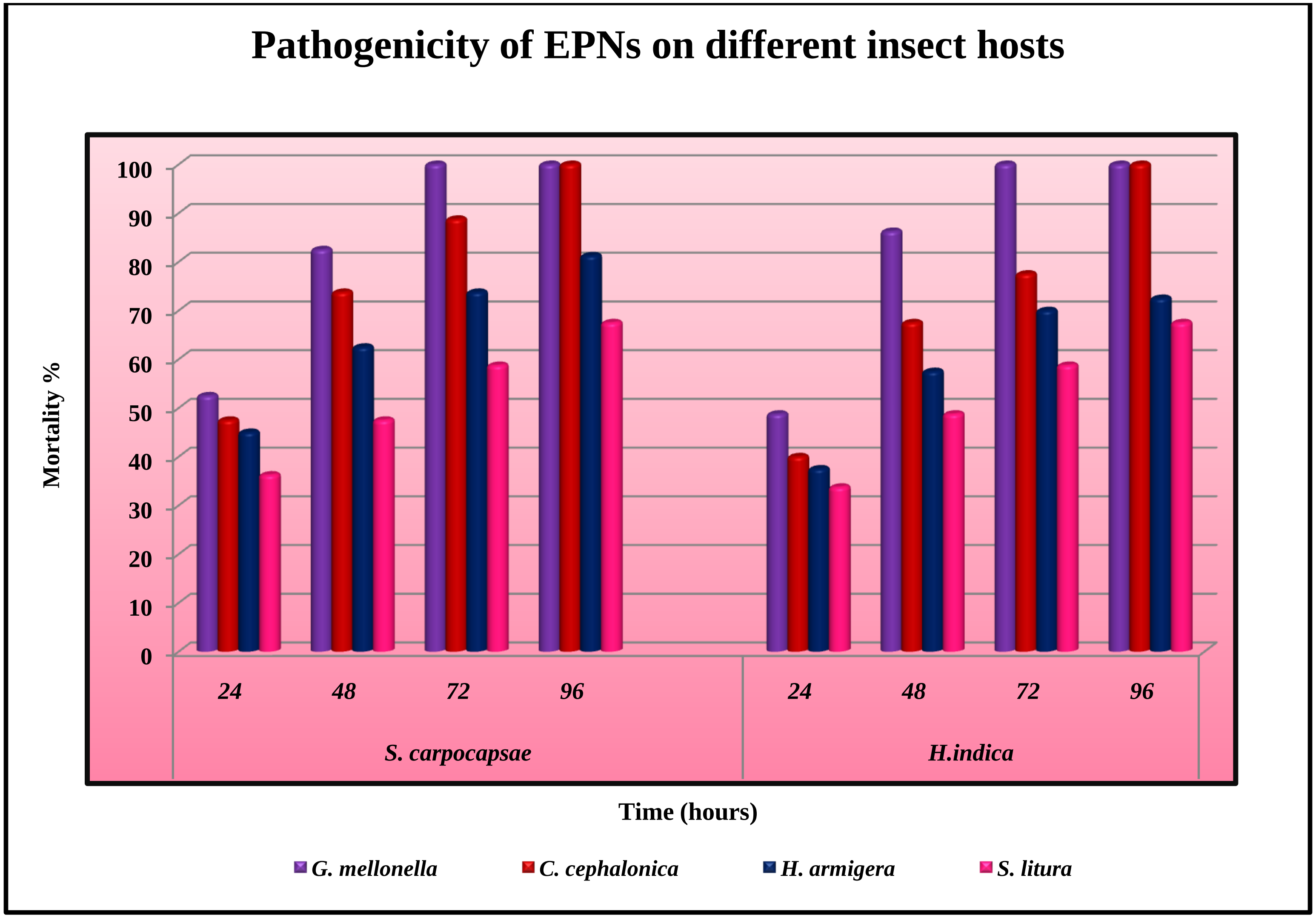



**Fig.4.34 Pathogenicity of EPNs on different insect hosts at different duration.**

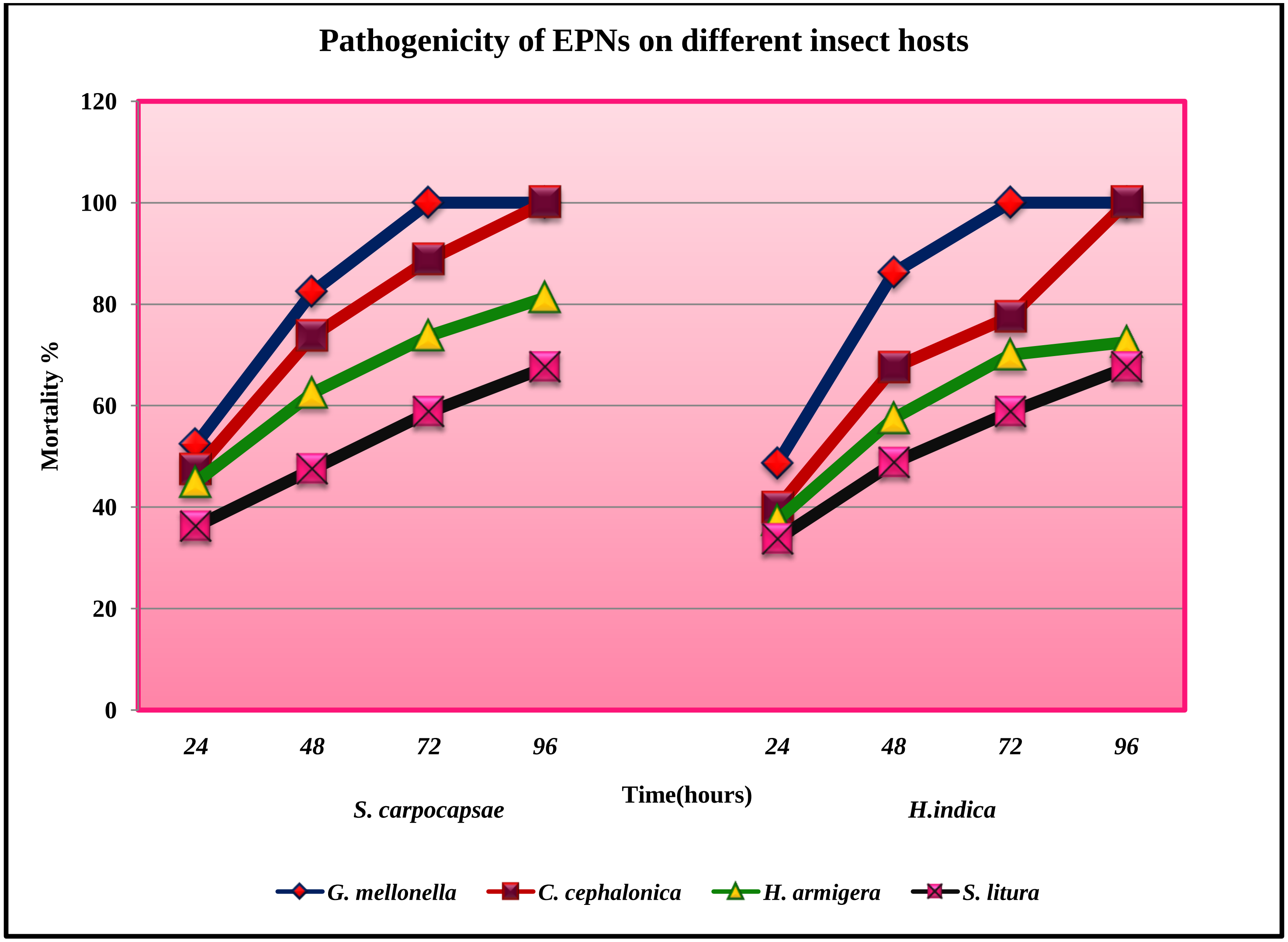



**Fig.4.35 Correlation of pathogenicity of EPNs on different insect hosts at different duration**


**Plate No. 18. EPNs infected cadavers (1. *S. carpocapsae* infected *G. mellonella* 2. *H. indica* infected *G. mellonella* 3. *H. indica* infected *C. cephalonica* 4. and 5. *S. carpocapsae* infected *C. cephalonica* 6. *S. carpocapsae* infected *H. armigera* cadaver)**

### 4.3.6 Progeny production of EPNs isolates from different species of insect hosts

Results pertaining to the influence of *S. carpocapsae* was found pathogenic to all the tested insect species such as *G. mellonella, C. cephalonica*, *H. armigera* and *S. litura,* indicated by the mortality that of larvae and progeny IJs produced from the cadaver. Highest progeny production leading to most preferred host as *G. mellonella* resulted in yielding 1,64,098.80 IJs/ larva followed by *C. cephalonica*, *H. armigera* and *S. litura* with 99,126.75, 88,609.75 and 77,849.25 number of IJs/ larva respectively, as presented in the Table No. 4.20.

Observations recorded to the influence of *H. indica* was also found pathogenic to all the test insect species such as *G. mellonella, C. cephalonica*, *H. armigera* and *S. litura,*, indicated by the mortality of the larvae and progeny produced from the cadaver. Highest progeny production pertaining to the with most preferred host as *G. mellonella* produced 3,42,264.00 IJs/ larva followed by *C. cephalonica, H. armigera* and *S. litura* with 1,22,181.30, 1,02,874.80 and 89,174.75 number of IJs/ larva respectively, as presented in the Table No. 4.20.

**Table No. 4.20 Progeny production of EPNs isolates from different insect hosts**

| S.No. | Host insect | Inoculum level (No. of IJs/ larva) | Progeny production of IJs/ larvae | |
|---|---|---|---|---|
| | | | ***S. carpocapsae*** | ***H. indica*** |
| **1.** | **Greater wax moth *G. mellonella*** | 60 | 1,64,098.80 (404.94) | 3,42,264.00 (584.98) |
| **2.** | **Rice meal moth *C. cephalonica*** | 60 | 99,126.75 (314.83) | 1,22,181.30 (349.31) |
| **3.** | **Gram pod borer *H. armigera*** | 60 | 88,609.75 (297.67) | 1,02,874.80 (320.64) |
| **4.** | **Tobacco caterpillar *S. litura*** | 60 | 77,849.25 (279.01) | 89,174.75 (298.61) |
| **5.** | **Control** | - | 0.00 (1.00) | 0.00 (1.00) |
| | **SE(m)** | - | 2.89 | 4.37 |
| | **C.D.(1%)** | - | 8.80 | 13.32 |

****Figures in parentheses are square root transformed value.**

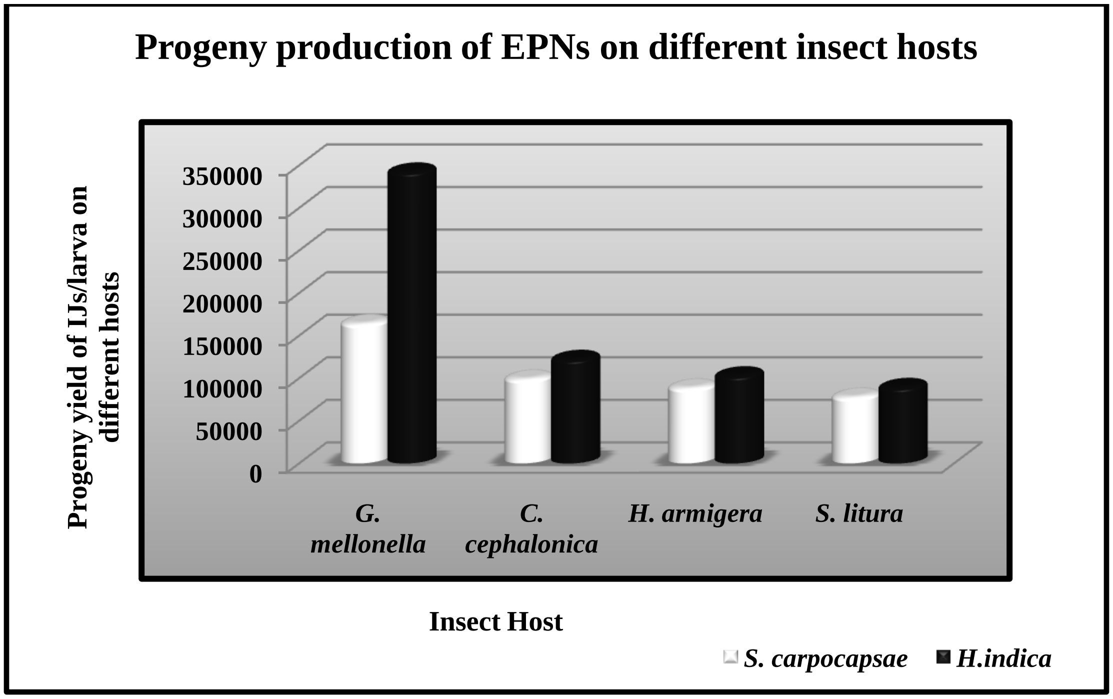



**Fig.4.36 Progeny production of EPNs on different insect hosts**

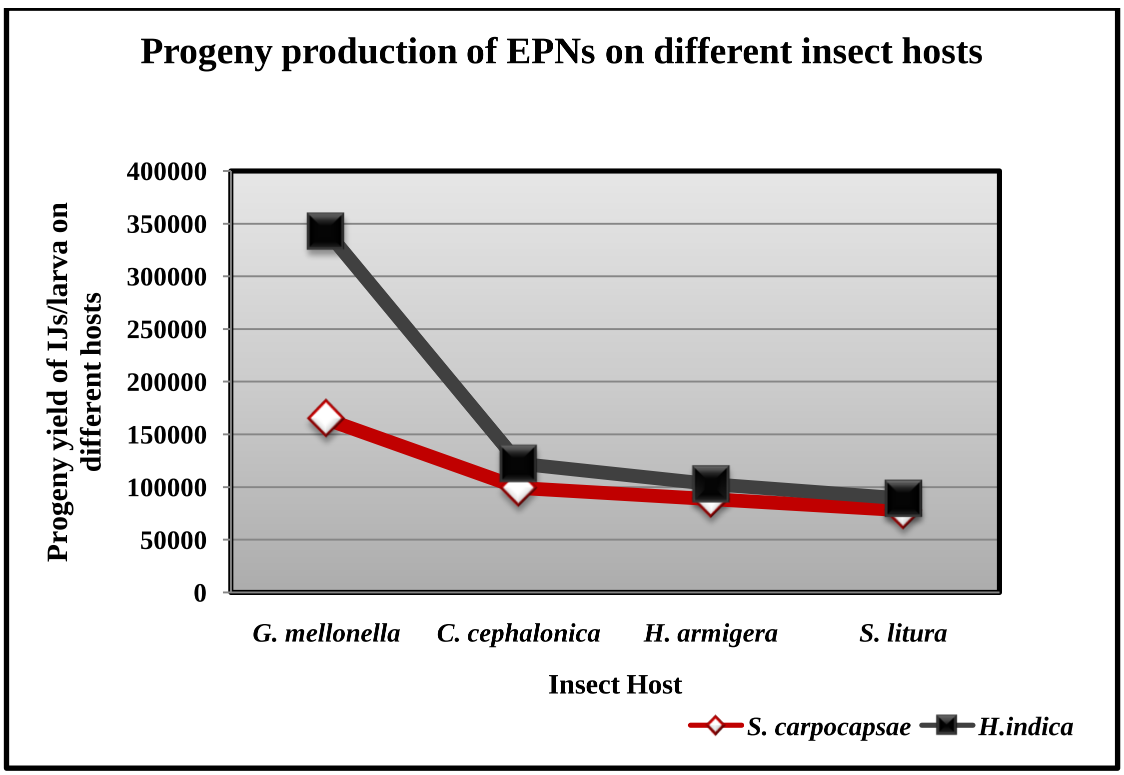



**Fig.4.37 Correlation of progeny production of EPNs on different insect hosts**

# CHAPTER – V

# SUMMARY AND CONCLUSION

The present investigation entitled, **"Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh"** was conducted in the region of Chhattisgarh plains and the Biological control laboratory, Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya Raipur (C.G.) during the year 2017-18. Findings of the investigation are summarized under the following objectives

**Objectives:-**

1. To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.
2. To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh.
3. To study the mass multiplication of EPNs at laboratory condition on different hosts.

**1. To isolate and identify entomopathogenic nematodes (EPNs) collected from different soil samples of respective agro-ecosystems under various geographical locations of Chhattisgarh.**

EPNs were isolated and identified from the soil samples collected from different districts under different agro-ecosystems belonging to the three agro-climatic zones of Chhattisgarh. Out of 56 soil samples of Chhattisgarh plains only two soil samples positively responded towards body discolouration of larvae and caused mortality within 48 hours. Body colour of larvae changed from greyish to black indicated the presence of IJs of *S. carpocapsae* and brick red colour indicated the presence of IJs of *H. indica,* collected from the samples of yam ecosystem at Raipur district and the other sample from rose ecosystem of Durg district. The confirmation of the presence of EPNs in the collected samples were confirmed from NBAIR, Bengaluru where, these two isolates of EPNs were identified on morphological basis.

**2. To study the ecological characterization of EPNs collected from different geographical locations of Chhattisgarh**.

Studies on the average effect of temperature on survival of the EPNs conducted under laboratory conditions revealed that the temperature of 25°C was found as the optimum temperature, which showed maximum of 99.60 per cent survival of the IJs of the *S. carpocapsae*. With increase in temperature from 30°C to 35°C, there was a sharp decrease in the percentage of survival from 82.93 to 44.93 per cent. Similarly, when temperature was decreased to 20°C, 15°C, 10°C and 5°C, the survival rates of IJs again decreased from 98.88, 98.36, 97.17 and 95.40 per cent respectively.

In case of *H. indica*, 99.95 per cent survival of IJs were recorded at the optimum temperature of 25°C. With increase in temperature from 30°C to 35°C there was a sharp decline in the percentage of survival from 87.33 to 46.40 per cent respectively. Similarly, when temperature was decreased to 20°C, 15°C, 10°C and 5°C survival rates of IJs again reduced but was lesser as compared to increase in temperature to 99.55, 96.61, 94.66 and 93.56 per cent respectively.

Observations recorded on the effect of U.V radiation on survival of *S. carpocapsae* revealed that more than 86.12 per cent of IJs could survive only up to 15 minutes of U.V radiation exposure (254 nm) and at a longer period of exposure of 120 minutes, resulted in no survival *i.e.* 100.00 percent mortality. In other treatments of U.V radiation exposure of 30.00, 45.00, 60.00, 75.00, 90.00 and 105.00 minutes, 75.00, 65.85, 54.25, 45.12, 32.25 and 15.50 per cent mortality was recorded respectively and in case of *H. indica* 82.37 per cent of IJs could survive only up to 15 minutes of U.V radiation exposure (254 nm) and at a longer period of exposure of 105 minutes, it resulted in 0.00 per cent survival *i.e.* 100.00 percent mortality of the IJs. In other treatments of U.V radiation exposure of 30.00, 45.00, 60.00, 75.00, and 90.00 minutes, reduction in percent survival viz., 64.25, 44.25, 29.00, 16.75 and 9.25 per cent was recorded respectively. Thus, it was clear that *S. carpocapsae* was comparatively tolerant towards U.V radiation for longer periods than *H. indica*.

The effect of U.V radiation on pathogenicity of *G. mellonella* larvae by using both the IJs of *S. carpocapsae* and *H. indica* revealed that among the two groups of EPNs, *S. carpocapsae* was more virulent in causing mortality of the test insect even when it was exposed to U.V radiation for 75 minutes as compared to *H. indica*. *S. carpocapsae* could sustain U.V radiation exposure up to 75 minutes causing 25.75 per cent mortality of the test insect, *G. mellonella* larvae where as at the same exposure duration, all the IJs of *H. indica* died, and could not cause any mortality. Hence, it can be concluded that among the two species of EPNs tested *S. carpocapsae* was more tolerant to U.V. radiation than *H. indica*.

The mean percentage survival of the IJs of *S. carpocapsae* at different pH revealed, a gradual increase in percentage survival of IJs of *S. carpocapsae* with increasing pH from 4.00 to 7.00. Highest survival per cent of the IJs of *S. carpocapsae* was found at neutral pH 7.00 *i.e.* 99.31 followed by 98.93 per cent at pH 9.00, followed by 98.75 per cent at pH 8.00 and lowest survival was found at pH 4.00 *i.e.* 96.43 per cent. In the case of *H. indica* there was a gradual decrease in percentage survival of IJs with increasing pH from 4.00 to 5.00, and highest survival was found at pH 5.00 *i.e.* 88.81 per cent followed by 85.50 per cent at pH 6.00, 81.93 per cent at pH 7.00 and lowest survival was found at pH 4.00 *i.e.* 62.12 per cent. From the above results, it was clear that at pH 7.00 and 5.00 was the optimum level for maximum survival of the *S. carpocapsae* and *H. indica* respectively.

**3. To study the mass multiplication of EPNs at laboratory condition on different hosts.**

Mass multiplication of both the species of EPNs namely, *S. carpocapsae* and *H. indica* in different lengths and body weight larvae of *G. mellonella* as test insect were conducted. Different length and body weight of *G. mellonella* ranged from 9-11 mm and 140-145 mg in small category 15–16 mm and 210-220 mg in medium category and 20–21mm and 300-315 mg in large category. Results revealed that the pooled mean production of IJs of *S. carpocapsae* yielded 94,311.66 IJs per larva of *G. mellonella*, whereas, in *H. indica* increased mean pooled production of 1,68,234.83 IJs per larva was recorded. Thus, the IJs of both *S. carpocapsae* and *H. indica* could be successfully cultured on all the three sizes of the test insect. However, there were significant differences on the number of IJs produced on different sizes of the larvae.

Mass multiplication of IJs of *S. carpocapsae* in different sized and body weight of larva of *C. cephalonica*, were tested ranging from 5 - 7 mm and 40-45 mg in small, 9–11 mm and 50-55 mg in medium and 15-17 mm and 70-80 mg in large sized and body weight of test insects resulted in a pooled mean production of of 84,484.83 IJs per larva. While the pooled mean production of IJs of *H. indica* yielded 1,09,094.83 IJs per larva. Thus, it can be concluded that IJs of both *S. carpocapsae* and *H. indica* could be successfully cultured on all the three sizes of test insect. However, there were significant differences on the number of IJs produced on different sizes of the larvae.

Studies conducted on the mass multiplication of both the species of EPNs *via., S. carpocapsae* and *H. indica* with different inoculums of IJs on *G. mellonella*, revealed that as the number of IJs of *S. carpocapsae* inoculum was increased, there was a significant increase in yield of the IJs. Highest progeny yield of 2,42,119.50 was recorded with inoculum of 60 IJs/ larva followed by 80 and 100 IJs producing a progeny yield of 2,34,240.50 and 2,00,983.30 numbers of IJs/ larva respectively. At minimum dosage of 20 IJs per larva, produced 1,20,957.00 numbers of IJs followed by 40 IJs producing 1,73,546.50 numbers of IJs. In case of *H. indica* highest progeny yield of recorded 4,82,084.50 numbers with inoculums of 80 IJs/ larva followed by 100 IJs producing 4,08,764.80 numbers of IJs/ larva. At dosages of 20, 40 and 60 IJs per larva, resulted in a mean progeny production of 3,24,269.50, 3,49,260.00 and 4,37,234.80 numbers of IJs/ larva respectively. Thus, lower inoculums doses of 60 IJs/ larva could yield maximum progeny in *S. carpocapsae* as compared to a higher dose of 80 IJs/ larva in case of *H. indica.*

Mass multiplication of both the species of EPNs such as *S. carpocapsae* and *H. indica* with different inoculums of IJs on *C. cephalonica* revealed that as the number of IJs in inoculum was increased, there was a significant increase in the progeny yield of the IJs as well. Highest progeny yield of 1,65,388.80 was recorded with an inoculum of 60 IJs/ larva followed by 80 and 100 IJs which producing a progeny yield of 1,51,429.00 and 1,35,668.50 numbers of IJs/ larva respectively. At minimum dosage of 20 IJs per larva resulted in a mean progeny production of 88,389.50 numbers of IJs followed by 40 IJs produced 1,25,530.30 number of IJs. In case of *H. indica,* highest progeny yield was recorded of 3,86,737.00 number an inoculum of 80 IJs/ larva followed by 100 IJs producing 3,24,942.80 number of IJs/ larva. At dosage of 20, 40 and 60 IJs per larva, resulted in a mean progeny production of 2,02,735.50, 2,28,027.30 and 3,52,599.80 numbers of IJs/ larva respectively.

Results presented regarding the pathogenicity of the two species of EPNs *i.e. S. carpocapsae* and *H. indica* against four lepidopteran larvae *i.e. G. mellonella, C. cephalonica, Helicoverpa armigera* and *Spodoptera litura* tested, depicted that both the species of EPNs were pathogenic to all the test insect species with most preferred host as *G. mellonella* resulting in significantly highest average mortality 83.75 per cent followed by *C. cephalonica,* with 77.50 per cent followed by *H. armigera* and *S. litura* with 65.62 and 52.50 per cent mortality respectively within 24-48 hours. There was no mortality in control in all the tested insect species.

*H. indica* was also found pathogenic to all the tested insect species with most preferred host as *G. mellonella* resulting in average mortality 83.42 per cent followed by *C. cephalonica,* with 71.25 per cent after that *H. armigera* and *S. litura* with 59.37 and 52.18 per cent mortality respectively within 24-48 hours. There was no mortality in control in all the tested insect species

Results pertaining to the progeny production of *S. carpocapsae* on *G. mellonella, C. cephalonica*, *H. armigera* and *S. litura* exhibited highest progeny production with most preferred host as *G. mellonella* resulting in an yield 1,64,098.80 IJs/ larva followed by *C. cephalonica*, *H. armigera* and *S. litura* as 99,126.75, 88,609.75 and 77,849.25 IJs/ larva respectively. In case of *H. indica,* highest progeny production was seen on *G. mellonella* which produced 3,42,264.00 IJs/ larva followed by *C. cephalonica*, *H. armigera* and *S. litura* with 1,22,181.30, 1,02,874.80 and 89,174.75 IJs/larva respectively.

## CONCLUSION

Thus, from the studies on **"Isolation, identification and mass multiplication of entomopathogenic nematodes (EPNs) at different districts of Chhattisgarh"** the followings conclusions can be drawn:

- Two species of EPNs *viz., S. carpocapsae* and *H. indica* were identified from yam ecosystem of Raipur and rose ecosystem of Durg out of 82 soil samples collected from different districts and various crop ecosystems of the three agro-climatic zones of Chhattisgarh.

- *S. carpocapsae* was isolated from yam ecosystem at Raipur district, while *H. indica* was recorded from the rose ecosystem of Durg district.
- Studies on the effect of temperature on survival of EPNs conducted under laboratory conditions revealed that, the temperature of 25°C was the optimum temperature, which showed a maximum of 99.60 per cent survival of the IJs of *S. carpocapsae*. Increase in temperature to 35°C was found to be unfavourable as it resulted in the lowest 44.93 per cent survival of the IJs. *H. indica* also exhibited maximum of 99.95 per cent survival of IJs at temperature of 25°C. Temperature of 35°C was found to be most unfavourable which resulted only 46.40 per cent survival of the IJs. However, for both the EPNs, temperature of 35°C was found to affect survival upto only 44.93 and 46.40 per cent in *S. carpocapsae* and *H. indica* respectively.
- In the studies on the effect of U.V. radiation on survival of the two species of EPNs, *S. carpocapsae* and *H. indica* maximum survival of 86.12 and 82.37 per cent recorded at 15 minutes of exposure to U.V radiation (254 nm). Exposure to longer periods 120 minutes, resulted in complete mortality of IJs of both the species.Thus, from the results it can be concluded that among the two species of EPNs, *H. indica* was found to be more sensitive to U.V radiation than *S. carpocapsae* and could comparatively resist U.V radiation for longer period than *H. indica*.
- As far as the effect of pH on survival of the IJs of *S. carpocapsae* was concerned, there was a gradual increase in percentage survival of IJs of *S. carpocapsae* with increasing pH from 4.00 to 7.00. Highest survival was found at pH 7.00 *i.e.* 99.31 per cent and lowest survival was found at pH 4.00 *i.e.* 96.43 per cent. In case of *H. indica* highest survival was found at pH 5.00 *i.e.* 88.81 per cent and lowest survival was found at pH 4.00 *i.e.* 62.12 per cent. Thus, from the above results, it can be concluded that at pH level of 7.00 and 5.00 the most suitable level for maximum survival of the *S. carpocapsae* and *H. indica* respectively.
- Looking to the progeny production of IJs, *S. carpocapsae* produced 94,311.66 IJs per larva and *H. indica* produced 1,68,234.83 IJs per larva in *G. mellonella* where as *S.*

*carpocapsae* produced lesser no. of 84,484.83 IJs and *H. indica* produced 1,09,094.83 IJs per larva in *C. cephalonica*.

- Mass multiplication of the two EPNs *i.e. S. carpocapsae* and *H. indica* was done using *G. mellonella* larvae that yielded highest progeny as 2,42,119.50 numbers IJs per larva when 60 IJs of *S. carpocapsae* were inoculated and lowest 1,20,957.00 numbers IJs per per larva recorded at inoculum levels of 20 IJs Whereas, in the case of *H. indica*, higher progeny production even with high inoculum level of 80 IJS per larva *i.e.* 4,82,084.50 IJs per larva was obtained. However, the same inoculum of *S. carpocapsae* could not yield high progeny under same conditions which may be due to the fact that its nutrients not could sustain more numbers of IJs/ larva.
- Mass multiplication of the two EPNs *i.e. S. carpocapsae* and *H. indica* using *C. cephalonica* larvae that yielded highest progeny 1,65,388.80 numbers IJs per larva when 60 IJs of *S. carpocapsae* were inoculated and lowest 88,389.50 at 20 IJs . In the case of *H. indica*, higher progeny production at an inoculum of 80 IJs per larva *i.e.* 3,86,737.00 numbers IJs per larva. However, the same inoculum of *S. carpocapsae* could not yield high progeny under same conditions. Thus, it was clear from the present studies that a single *C. cephalonica* larva could accommodate only 60 IJs of *S. carpocapsae* and 80 IJs of *H. indica* for mass multiplication. More than 60 IJs of *S. carpocapsae* and 80 IJs of *H. indica* yielded low progeny.
- Estimation of progeny production of the EPNs tested on different insect hosts *i.e. G. mellonella, C. cephalonica*, *H. armigera* and *S. litura.* revealed that *G. mellonella* yielded highest progeny of *S. carpocapsae* 1,64,098.80 IJs/ larva followed by *C. cephalonica* with 99,126.75 IJs per larva and lowest of 88,609.75 and 77,849.25 IJs/ larva in *H. armigera* and *S. litura*. Similar pattern of progeny yield was recorded with respect to *H. indica i.e. G. mellonella* yielded high population of 3,42,264.00 IJs/ larva followed by 1,22,181.30 IJs/ larva from *C. cephalonica*, where as lowest of 1,02,874.80 and 89,174.75 IJs/ larva in *H. armigera* and *S. litura*. Thus, it can be concluded from the results that *G. mellonella* was the best host that could be used for mass production of *S. carpocapsae* and *H. indica* and *C. cephalonica* can be the alternate host in the absence of *G. mellonella*

## SUGGESTIONS FOR FUTURE WORKS

1. More intensive survey of soil samples collection should be done focusing summer, winter and rainy months of Kharif and Rabi season.
2. Studies on horizontal movement of EPNs should also be done.
3. Soil related parameters should also analyzed and correlated with the abundance of nematodes.

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