BIO-ECOLOGY OF ENTOMOPATHOGENIC NEMATODES

By

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CERTIFICATE – I

This is to certify that this thesis entitled "**Bio-ecology of Entomopathogenic Nematodes**" submitted for the degree of Master of Science in the subject of Nematology of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bona-fide research work carried out by **Mr. Vivek Narayan Sutar**, Admn. No. 2011A81M under my supervision and that no part of the thesis has been submitted by him for any other degree. All the assistance and help received during the course of investigation have been duly acknowledged.

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CERTIFICATE – II

This is to certify that this thesis entitled "**Bio-ecology of Entomopathogenic Nematodes**" submitted by **Mr. Vivek Narayan Sutar,** Admn. No. 2011A81M to the Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfillment of the requirements for the degree of Master of Science in the subject of Nematology, has been approved by the Student's Advisory Committee after an oral examination on the same.

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CHAPTER-I

INTRODUCTION

There is an increasing demand by governments today for safe pesicides with low toxicity, short term persistence, low mobility in the soil to avoid ground water contamination and limited effects on non-target organisms. These concerns about environmental health and safety have led to increased restrictions on a variety of chemical pesticides (Ehlers, 1996; Strauch and Ehlers, 1998). The growing concern about chemical residues, worker's safety and shifting cosumer demands favour the adoption of more environmental friendly management tools for insect pests (Rodriguez *et al*., 2006).

Entomopathogenic nematodes (EPNs) are efficient biocontrol agents since they kill a broad range of insect hosts within 24-48 h of application (Gaugler,1998; Hazir *et al*., 2003; Rodriguez *et al*., 2006), can be applied easily using standard spray equipments (Georgis and Kaya, 1998; Hayes *et al*., 1999) and can tolerate or can be applied along side many types of pesticides (Nishimatsu and Jackson, 1998; Koppenhofer *et al*., 2000). In addition, their effects are non-detrimental to non- target organisms including mammals, plants (Kaya and Gaugler,1993) and human beings (Boemare *et al*., 1996; Akhurst and Smith, 2002).

In almost all countries, EPNs are exempted from registration requirements as bio-control agents of insect pests (Kaya and Gaugler,1993). This enables small and medium sized enterprises to develop nematode based plant protection products (Ehlers, 2001). EPNs are mainly produced commercially in Asia, Europe and USA by very few companies. As a result, the prices of EPNs are still high to permit their application on low value crops (Kaya and Grewal, 2002).

EPNs belonging to the families Steinernematidae and Heterorhabditidae are highly pathogenic to soil dwelling insects. They are found under diverse ecological conditions. Substantial efforts have been made in EPN research to isolate, identify and test range of pathogenicity against economically important insect pests. In the broad geographic sense, EPNs are wide spread throughout the world except Antarctica (Hominick *et al*., 1996; Hominick, 2002; Kaya *et al*., 2006).

Any biocontrol agent is found more effective if isolated from the site where it is to be used against the target pest since it will be well adapted to the prevailing environmental conditions (Bedding,1990). The edaphic and other environmental factors do affect the diversity and distribution of EPN species. Many workers have studied the effect of these

environmental factors on occurrence of EPNs in different agro-climatic ecosystems (Prasad *et al*., 2001b; Mracek *et al*., 2005;Campos-Herrera *et al*., 2007; Mwaniki *et al*., 2008). Soil pH, longitude, available water and altitude determine the presence and absence of EPNs. The EPNs occur frequently in undisturbed ecosystems with acidic (pH 3.7 to 7.0), more sandy textured and sufficiently moisturised soils (Kanga *et al*., 2007).

The life cycle of EPNs from entry of IJs into the host until emergence of new IJs is dependent on temperature and varies among species and strains (Hazir *et al*., 2001). It generally takes approximately 6-18 days at temperature ranging from 18 to 28° C in *Galleria mellonella* (Poinar, 1990; Nguyen and Smart, 1992). However, to overcome extreme cold conditions during winters, the EPNs do employ some survival strategies to avoid the cold stress and remain infective when favourable conditions return. Steinernematids can survive freezing temperatures (Molyneux, 1984, 1986; Brown and Gaugler, 1996;) and heterorhabditids show infectivity in the 6-12° C range (Griffin and Downes, 1991). These studies have been carried out on temperate species and thermal requirements for species isolated from tropical and sub-tropical regions may vary. The air temperature fluctuates between 0° C in winters to 48° C in summers in the southern part of the Haryana state (India). The temperature in soil at 10 cm depth is not as high as air temperature and hovers around 30-35° C maximum. How these temperatures affect the life processes of EPNs formed a part of present studies.

Storage of IJs of EPNs for long periods is not easy. Aqueous suspension of IJs can be stored for up to two months in tissue culture flasks at 5-15° C with proper aeration. It has experimentally been demonstrated by various workers that host cadaver provides protection to IJs for nematode development in sand (Perez *et al*., 2003) and under environmental stress conditions of freezing temperature and desiccation (Koppenhofer *et al*., 1997; Lewis and Shapiro-Ilan, 2002). Better infectivity and dispersal of IJs from host cadavers than in aqueous suspension (Shapiro-Ilan and Glazer, 1996; Shapiro-Ilan and Lewis, 1999) has led to development of host cadaver-based formulation (Shapiro-Ilan *et al*., 2001). A variety of formulations are available to facilitate nematode storage and appliction (Ehlers, 2001). These include charcoal, alginate and polyacrylamide gels, baits, clay, vermiculite and water dispersible granules (WDG) of which WDG have the greatest shelf life (6 months). Host cadaver-based formulation has an advantage over others that IJs can survive dry conditions for extended periods if they remain inside a host cadaver (Koppenhofer *et al*., 1997).

The success of EPNs and their use as successful biocontrol agents is hampered due to their sensitivity to some ecological and behavioural factors. It is important for their success that they are able to overcome these hurdles efficiently. This ability may result in long-term persistence of an EPN Meta population (Stuart *et al*., 2006). Hence, it is essential to study the impact of the environmental stresses on the infectivity and survival of EPNs; and consider

information in field application of EPNs against insect pests of crops. In view of the aforementioned, the current study sought to determine the storage potential of EPNs in various media at various temperatures, the effect of temperature on life cycle of EPNs inside host cadavers and their compatibility with different recommended novel insecticides, with the following objectives.

1. To compare the EPN fauna of disturbed and undisturbed ecosystems.
2. To study the effect of low temperature on development and infectivity of EPNs inside host cadavers.
3. To assess the storage of EPNs inside host cadavers.
4. To ascertain the compatibility of EPNs with selected insecticides for field applications.

CHAPTER-II

REVIEW OF LITERATURE

The field of entomopathogenic nematology has experienced exponential growth over the past decade. A hundred different laboratories explore these nematodes and their bacterial symbionts in more than 60 countries from every inhabited continent. Despite research breadth that extends from molecular biology to field ecology, the discipline is unified by a common interest in biological control.

However, use of EPNs as successful biocontrol agents is hampered due to their sensitivity to some ecological and behavioural factors. It is important for their success that they are able to overcome these hurdles efficiently. Hence, it is essential to study the impact of the environmental stresses on the infectivity and survival of EPNs; and consider information in field application of EPNs against insect pests of crops. In this chapter, the available recent information on the topics of study has been put together while excellent reviews/books by Gaugler and Kaya (1990), Kaya (1990), Gaugler (2002), Lewis (2002) and Hussaini *et al*. (2003) form the basis of this information.

The first entomopathogenic nematode was described by Steiner as *Aplectana kraussei* (now *Steinernema kraussei*) in 1923. The symbiotic bacterium (under the name *Achromobacter nematophilus*) associated with *S. carpocapsae* was described by Poinar and Thomas (1965). The genus *Heterorhabditis* was described Poinar (1976) and the symbiotic bacterium of *H. bacteriophora* was characterized as *Xenorhabdus luminescence* in 1979 by Thomas and Poinar.

2.1. Distribution and occurrence of EPN fauna in disturbed and undisturbed ecosystems

The EPNs are wide spread in tropical, subtropical and temperate areas globally except Antartica (Hominick, 2002). Occurrence and distribution of EPNs has been studied in various local geographical locations (Mracek *et al*., 2005; Campos-Herrera *et al*., 2007; Mwaniki *et al*., 2008). In India too the EPNs have been reported in all the ecological zones (Hussaini, 2003). *H. indica* was reported from India in 1992 by Poinar *et al*., since then many species of *Steinernema* particularly have been reported of which some are new to science (Ganguly and Singh, 2000; Prasad *et al*., 2001a; Ganguly *et al*., 2011).

Mracek *et al.* (2005) reported that nematodes occurred in all ecosystems and habitats tested. They were more abundant in tree habitats and light soils and in sites with abundant

suitable insect hosts; seasonality and altitude had no significant impact on their occurrence. At two laboratory temperatures (15 and 22° C) different numbers of isolates were obtained from the *Galleria* bait traps. Abundance of EPNs in soil samples varied considerably.

Alumai *et al.* (2006) reported that 'surface type' was the most important factor in predicting EPNs occurrence in golf courses. Putting greens differed significantly from fairways and rough areas in the number of EPN-positive sites. No EPNs were recovered from putting greens but were recovered from 43 per cent of the fairways and 57 per cent of the rough areas. Putting greens also differed significantly from fairways and rough areas in organic matter, pH, calcium, and phosphorus. The fairways and rough areas did not however differ in number of EPN-positive sites and measured soil parameters. Presence of EPNs correlated significantly, although weakly, with sand, silt, phosphorus, organic matter, and magnesium content, but not with clay, pH, calcium, and potassium. This suggests that EPNs are more likely to occur in less intensively managed sites that receive fewer inputs and have relatively high sand, and moderate silt, organic matter, phosphorus, and magnesium content.

A total of 440 sites across four habitats (cultivated areas, grassy banks adjacent to cultivated areas, undisturbed shrub lands and forests) associated with the production landscape were sampled to ascertain the natural occurrence of EPNs. Twelve sites along grassy banks were monitored over a growing season to estimate associations between abiotic and biotic factors and endemic populations. EPNs were only detected along grassy banks adjacent to the cultivated areas; nematodes were recovered from 15 and 30 per cent of sites sampled in 2001 and 2002, respectively. Two species of nematodes were isolated, *Heterorhabditis bacteriophora* and *Steinernema feltiae*. *H. bacteriophora* was the most prevalent nematode species and was recovered from 60 per cent of positive samples. Nematode populations varied temporally and spatially along grassy banks. Neither macro- nor microarthropod communities nor soil temperature differed between sites at which nematodes were detected and those at which nematodes were not detected. Soil moisture, however, was associated with the occurrence and persistence of nematodes along grassy banks; mean soil moisture at sites at which nematodes were detected and those sites at which nematodes were not detected was 37.3 and 26.8 per cent, respectively. Water management is an important component of vegetable production and results suggest that soil moisture manipulation would be important in the establishment and sustained presence of EPN populations within cultivated areas over the growing season (Lawrence *et al.*, 2006).

KoppenhÖfer and Fuzy (2006) tested the effect of soil type on the performance of *Steinernema scarabaei*, *S. glaseri*, *H. zealandica*, and *H. bacteriophora*. Soil types used were loamy sand, sandy loam, loam, silt loam, clay loam, acidic sand, and a highly organic potting mix and they found that infectivity of *H. bacteriophora* and *H. zealandica* was the highest in potting mix, did not differ among loamy sand and the loams, and was the lowest in

acidic sand. Infectivity of *S. glaseri* was significantly lower in acidic sand than in loamy sand in a laboratory experiment but not in a greenhouse experiment, and did not differ among the other soils. Infectivity of *S. scarabaei* was lower in silt loam and clay loam than in loamy sand in a green-house experiment but not in a laboratory experiment, but was the lowest in acidic sand and potting mix. Persistence of both *Heterorhabditis* spp. and *S. glaseri* was the shortest in potting mix and showed no clear differences among the other substrates. Persistence of *S. scarabaei* was high in all substrates and its recovery declined significantly over time only in clay loam.

Campos-Herrera *et al.* (2007) reported that, there were no statistical differences ($p \leq 0.05$) in the abundance of EPNs to environmental and physico-chemical variables, although, there were statistical differences in the altitude, annual mean air temperature and rainfall, potential vegetation series and moisture percentage recovery frequency. Twenty-seven samples from 14 sites were positive for EPNs. From these samples, twenty isolates were identified to a species level and fifteen strains were selected: 11 *S. feltiae*, two *S. carpocapsae* and two *S. kraussei* strains. *S. kraussei* was isolated from humid soils of cool and high altitude habitats and *S. carpocapsae* was found to occur in heavy soils of dry and temperate habitats. *S. feltiae* was the most common species with a wide range of altitude, temperature, rainfall, pH and soil moisture, although this species preferred sandy soils.

Moisture ranges for infectivity and persistence vary among species. Differences among species may be based on differences in size and behavioural and physiological adaptations. Nematode infectivity was highest at moderate soil moistures (−10 to −100 kPa), and tended to be lower in wet (−1 kPa) and moderately dry (−1000 kPa) soils. Highest activity was wider in loamy sand (−1 to −1000 kPa) than in loamy sand and silt loam (−10 to −100 kPa). Persistence was determined in laboratory by baiting nematode-inoculated soil with larvae of *G. mellonella*. For both, *H. bacteriophora* and *H. zealandica* persistence was short at −10 kPa, improved slightly at −100 kPa, significantly at −1000 kPa, and was the highest at −3000 kPa. Both *Steinernema* spp. persisted very well at −10 kPa. However, *S. glaseri* persistence was the shortest at −10 kPa but did not differ significantly at −100 to −3000 kPa, whereas *S. scarabaei* persistence was not affected by soil moisture (KoppenhÖfer and Fuzy, 2007).

Abiotic factors also have strong effects on EPN persistence; especially for vulnerable free-living stages. A year-long experiment showed EPN mortality was weakly correlated with soil moisture (Karthik *et al.*, 2008).

Campos-Herrera *et al.* (2010) reported that, the highest occurrence of EPNs was in autumn, followed by spring and summer. Agricultural management influenced the amount of soil organic matter, nutrients and heavy metals, as well as soil volumetric moisture

and temperature, leading to statistical differences mainly between natural and agricultural sites, but also in some cases between different types of agricultural management.

Recently, EPNs have been reported from southern Cameroon in three ecological zones (Kanga *et al*., 2012). *Heterohrabditis baujardi* was the most common (88.5% frequency) species. Also, soil type, soil pH, longitude, available water and altitude were associated with presence and absence of EPNs.

2.2. Effect of low temperature on development and infectivity of EPNs inside host cadavers

EPNs survive freezing temperatures inside their host and go on to reproduce once moderate temperatures resume. Brown and Gaugler (1996) observed that IJs of many EPN species are freeze tolerant, while Wharton and Surrey (1994) reported *H. zealandica* employing freeze avoiding strategy. KoppenhÖfer *et al*. (1997) suggested that EPNs remain inside their host cadaver during dry soil conditions which resulted in extended survival during harsh environmental conditions.

Menti *et al*. (2000) reported that the temperature range for infectivity was greater than that for development. However, the optimal temperature for infection and development for all populations was 23° C. Infectivity of *Steinernema* populations was not affected by storage for 12 weeks. However, 12 week-old *H. megidis* UK 211 IJs were less infective than fresh IJs. *H. megidis* GR showed very low establishment rates at all the temperatures tested, before and after storage.

2.3. Storage of EPNs inside host cadavers

EPNs can be stored effectively after formulating in gels, wettable powder, vermiculte sponge, clay, alginate capsules and water dispersible granules for various periods (Grewal and Peters, 2005). Formulations in inert carriers like vermiculite and sponge can be stored for 1 to 4 months but under refrigeration (2-10° C), since nematodes are active in these formulations and tend to move out. Calcium alginate gels, flowable gels reduce the activity (Georgis, 1990; Grewal, 2002). Nematodes have also been formulated in various heteropolysachharides (agarose, guar gum, carbopol, etc.) surrounded by a paste of hydrogenated oil. Up to 35 days of storage of *S. carpocapsae* at room temperature was obtained in this formulation (Chang and Gehert, 1995). Water dispersible granular formulation of *S. carpocapsae* developed by Grewal and Georgis (1998) has a shelf life of six months but is prone to microbial contamination.

Strauch *et al*. (2000) tested nematode survival at temperatures ranging between 5-25° C. A maximum survival of *H. indica* was achieved at 15° C and the highest mortality at 5° C. *H. bacteriophora* survived best at 7.5° C and least at 2 - 5° C. Infectivity was not influenced by the different formulation materials. When stored in sponge at 25° C, nematodes survived less than one week and the formulation in clay could only prolong this

period for another week. At 5° C, the survival of *H. bacteriophora* in sponge was superior to that in clay, whereas *H. indica* survived less well in sponge than in clay at 15° C.

Host cadaver-based formulation has been developed by Shapiro-Ilan *et al*. (2001); cadavers can be coated with a protective formulation to prevent rupturing during storage and shipping.

S. carpocapsae, *S. riobrave*, or *H. bacteriophora* were used to compare survival among IJs emerging in water from hosts in White traps, emerging in sand from hosts placed in sand, and emerging from hosts placed on a mesh suspended over sand. Nematode survival was recorded in sand at three-day intervals during 21 days of storage in desiccators at 75 per cent relative humidity and 25° C. Treatment did not affect per cent survival of *H. bacteriophora* IJs. Per cent survival of *S. riobrave* and *S. carpocapsae* IJs was lowest in White trap. Across all treatments, by 10 days after the beginning of the experiments, IJ survival declined to 93, 43, and 28 per cent of levels on day one for *H. bacteriophora*, *S. riobrave* and *S. carpocapsae*, respectively (Perez *et al.*, 2003).

Chen and Glazer (2005) showed that after storage for six months at 23° C, the survival rates of nematodes in the granules in the desiccator at 100 per cent RH and in the plastic box were 99.8 ± 0.4 and 95.9 ± 4.3 per cent, respectively. Under the same conditions, the survival rates of nematodes stored in distilled water and in granules without osmotic solution were 10.3 ± 1.9 and 14.7 ± 4.7 per cent, respectively. EPNs stored in the desiccator at 85 per cent RH were all dead after four months.

Deol *et al.* (2011) compared survival of *S. carpocapsae,* delivered via *G. mellonella* and *Tenebrio molitor* cadavers in the Scotts commercial growing medium, Miracle-Gro®. The nematode survival was assessed in the cadavers, pouches and the bags. Although, overall survival of *S. carpocapsae* declined within 90 days in both cadaver types, survival was significantly higher in *G. mellonella* than *T. molitor* cadavers at 15, 45, 60 and 90 days most likely due to the higher reproduction in *G. mellonella* cadavers. Similarly, survival (assessed as the number of baited *G. mellonella* killed) remained consistently higher both in the pouches and bags in the *G. mellonella* cadaver treatment compared to the *T. molitor* treatment. In another experiment in which bags were not opened until 180 days, all *G. mellonella* cadavers disintegrated within 180 days of storage; however, the number of baited *G. mellonella* larvae killed in the pouch was similar in both the cadaver treatments indicating equal number of surviving nematodes. Further, they evaluated the effect of initial moisture content of the growing medium on the survival and virulence of nematodes when delivered through cadavers and aqueous suspension. The results showed that survival and virulence of *S. carpocapsae* was higher in *G. mellonella* cadaver treatment than in the aqueous suspension treatment at all sampling times and reducing the initial

moisture content of the medium from 50 to 20-30 per cent enhanced nematode survival in the medium.

2.4. Compatibility of EPNs with selected insecticides

Cuthbertson *et al.* (2003) studied the effects of direct exposure for 24 h to field rate dilutions of four insecticides (imidacloprid, buprofezin, teflubenzuron and nicotine) on nematode infectivity to *G. mellonella* larvae in a sand tube bioassay. Teflubenzuron had the least adverse effect on the nematodes whilst imidacloprid, buprofezin and nicotine significantly reduced nematode infectivity. The impact of dry insecticide residue present on tomato and verbena foliage on nematode infectivity against *Bemisia tabaci* larvae was investigated for buprofezin, teflubenzuron and nicotine (imidacloprid is a systemic pesticide and no residues would occur on foliage). No significant reduction on the level of control of *B. tabaci* was recorded when compared with the infectivity of nematodes applied to residue-free foliage of either tomato or verbena plants. Nematodes in combination with imidacloprid gave significantly higher *B. tabaci* larvae mortality compared to either treatment individually on both host plants.

Alumai and Grewal (2004) found that *S. carpocapsae* viability was not affected by any of the pesticides, while aluminum tris and trichlorfon significantly reduced *S. carpocapsae* pathogenicity at all concentrations. Thiamethoxam and trichlorfon significantly reduced *H. bacteriophora* viability, while halofenozide, aluminum tris, trichlorfon, and carbaryl significantly reduced *H. bacteriophora* pathogenicity. Imidacloprid, at the recommended rate 330-440 g a.i. per ha, significantly increased *H. bacteriophora* pathogenicity at 500 and 300 l per ha.

The compatibility of three species of EPNs (*H. indica*, *S. carpocapsae* and *S. glaseri*) and 18 insecticides registered to control *Spodoptera frugiperda* in corn crop was evaluated by observing mortality and infectivity of IJs 48 h after immersion in the insecticide formulations. Among all insecticides tested chlorpyrifos, deltamethrin, lufenuron, deltamethrin + triazophos, diflubenzuron, gamacyhalothrin, lambdacyhalothrin, spinosad, chlorpyrifos, cypermethrin, triflumuron, and permethrin were compatible with the three nematode species tested under laboratory conditions (Negrisoli *et al.*, 2010).

Aqueous solutions of five pesticides (acephate, bifenthrin, deltamethrin, fipronil, and imidacloprid) used in turfgrass to control mole crickets were tested for compatibility with *Steinernema scapterisci* in the laboratory. Survival of *S. scapterisci* was > 95 per cent in solutions of acephate, bifenthrin, and imidacloprid. Infectivity of *S. scapterisci* in adult *Scapteriscus vicinus* was > 60 per cent in acephate and bifenthrin; however, infectivity was < 40 per cent in imidacloprid. The nematode was compatible with most insecticides tested without significantly reduced survival or infectivity (Kathryna and Elieen, 2005).

García del Pino and Jové (2005) assessed the survival and infectivity of IJs of three species of EPNs, *S. carpocapsae*, *Steinernema arenarium* and *H. bacteriophora*, after exposure to different concentrations (250, 500, 1000 and 2000 ppm) of fipronil. *H. bacteriophora* was very tolerant to all concentrations of fipronil, with the highest mortality of 17 per cent being observed at 2000 ppm of fipronil after 72 h exposure. *S. carpocapsae* showed a similar response, with the highest mortality of 11.25 per cent of IJs being observed after 72 h exposure to 2000 ppm of fipronil. *S. arenarium* was, however, more sensitive to fipronil, and at 2000 ppm mortality rates of 94.6 and 100 per cent were observed after 24 and 72 h, respectively. Fipronil had negligible effects on the infectivity of the three nematode species tested. The IJs which survive exposure to all concentrations of fipronil tested can infect and reproduce in *Galleria* larvae.

Gutierrez *et al.* (2008) evaluated the effect of three neurotoxic insecticides, three photosynthetic inhibitor herbicides and three enzymatic inhibitor herbicides on IJs of *S. feltiae* Rioja (native) and ENTONEM® (commercial) strains were evaluated after 48 h exposure at field tank concentrations and overnight treatment in mQ-water, using *Spodoptera littoralis* as target. Nematode survival was not affected by acetyl-cholinesterase inhibitors, chlorpyrifos and pirimicarb, although chlorpyrifos seriously reduced their virulence. Both nematode strains showed differential sensitivity to cypermethrin, with the ENTONEM® strain being more tolerant than Rioja strain. However, these chemicals showed a strong sub-lethal effect on the nematode reproductive potential, limiting seriously their possible recycling in the field. Herbicides showed differential toxic effects on nematode survival. The commercial strain was tolerant to enzymatic inhibitor herbicides, whereas tribenuron and chlorsulfuron reduced Rioja strain survival. However, photosynthetic inhibitor herbicides severely affected survival of both nematode strains, with the Rioja strain being more sensitive. Sub-lethal effects on both nematode strains were observed only after exposure to terbutryn + chlortoluron + triasulfuron, increasing the time to kill insect larvae.

The survival and infectivity of the IJs of two species of EPNs, *S. feltiae* and *H. bacteriophora* were determined after exposure for 72 h to two concentrations of the herbicides glyphosate and MCPA, as well as to the combination of the two herbicides (glyphosate + MCPA). For all herbicide treatments, concentrations and exposure times, *S. feltiae* was more tolerant to the herbicides than *H. bacteriophora*. The exposure of nematodes to glyphosate + MCPA caused significantly higher mortality (26.33-57.33%) than glyphosate (0.67-15%) or MCPA (2.33-19%) alone. These results confirm the synergistic effect of the glyphosate + MCPA combination on the mortality in these nematodes. Nematode infectivity of *G. mellonella* larvae in response to the herbicides presence was evaluated in Petri dish assays containing sterile sand. Nematode infectivity was not significantly reduced by exposure to herbicides in *S. feltiae* but *H. bacteriophora* was less

tolerant. Synergistic effect was obtained in the nematode mortality test but no synergistic effect was observed in the nematode infectivity assay (Garcia del Pino and Morton, 2010).

The effect of spray adjuvants viz., Sunflower oil, Glycerol, Tween 20, Triton X-100 and Paraffin liquid at 1 per cent concentration on survival and infectivity of indigenous EPNs (*S. carpocapsae* and *H. indica*) of Meghalaya was evaluated. Triton X-100 and Tween 20 showed little toxicity and Paraffin liquid and Sunflower oil showed higher toxicity against the IJs of *S.carpocapsae* (EPN-S-V) and *H. indica* (EPN-H-II). Infectivity of adjuvant-exposed (Triton-X 100, Tween 20 and Glycerol) IJs of both the isolates was not adversely affected against larvae of *G. mellonella* (Devi, 2011).

Pervez and Ali (2012) studied the effect of aqueous suspension of the insecticides (endosulfan and monocrotophos), fungicide (mancozeb), weedicide (pendimethalin) and botanical (nemmarin) on the activity of IJs of *Steinernema masoodi, Steinernema seemae, S. carpocapsae* and *Steinernema mushtaqi* and infectivity of IJs pre-exposed to these pesticides against *Corcyra cephalonica* larvae. *S. mushtaqi* was found more compatible with all tested pesticides followed by *S. masoodi* and *S. seemae*. However, *S. carpocapsae* was found least compatible with tested pesticides. Immobility of IJs increased with increase in the exposure period. Maximum number of inactive juveniles was recorded after 72 h exposure. Among the tested pesticides, endosulfan was more effective on the activity of the juveniles followed by monocrotophos, while nemmarin showed less effective on the activity of the IJs. The infectivity of 24 h pesticides exposed IJs was not much affected against *C. cephalonica* larva as compare to control. Among the tested pesticide, IJs of all EPN species showed less infectivity when exposed to endosulfan followed by monocrotophos. However, pendimethalin and nemmarin exposed IJs were found more pathogenic to rice moth larvae.

CHAPTER-III

MATERIALS AND METHODS

3.1 Mass rearing of insects and entomopathogenic nematodes

3.1.1 Preparation of artificial diet

Artificial diet (Plate 1) was prepared by mixing various components in proportions as given below:

Maize flour	4 parts
Wheat flour	2 parts
Wheat bran	2 parts
Milk powder	2 parts
Yeast extracts	1 part
Honey	200 ml
Glycerine	200 ml

First maize flour, wheat flour, wheat bran, milk powder and yeast extract were mixed properly in a clean pan. Honey and glycerine were mixed separately in a beaker thoroughly. This mixture was added slowly to pan and mixed well, taking care that no lumps are formed and the flour mixture is not too wet. The diet was stored in an air tight container and kept in refrigerator at low temperature overnight before use.

3.1.2 Greater wax moth, *Galleria mellonella L.*

Stock culture of *G. mellonella* (Plate 2) was obtained from bio-control laboratory of Department of Nematology, CCS HAU, Hisar. *G. mellonella* larvae were maintained in a glass jar on artificial diet. Simultaneously, moths were collected and kept in another glass jar containing cotton soaked in 15% honey or sucrose solution and paper folded in zig-zag manner for oviposition. After 3-4 days, eggs on paper were transferred to the glass jar containing small amount of artificial diet. Insect culture was maintained at 25±1° C in a BOD incubator. Last instar larvae were used whenever required during the course of the present investigations.

3.1.3 Entomopathogenic Nematodes (EPNs)

Different strains and/or species of EPNs (*Heterorhabditis* spp.) maintained in laboratory were mass reared by inoculating the IJs on filter paper fitted in lid of Petri plate @ 20 IJs per *Galleria* larva.

Plate 1: Artificial diet for raising
Greater wax moth, *Galleria mellonella* culture

Plate 2: *Galleria mellonella* larvae on artificial diet

Galleria was dead within 24-72 h. The cadavers were transferred on dry filter paper in Petri plate and kept covered in polythene bags in an incubator at 25 ±1° C for 8-12 days for *Heterorhabditis* spp. Within this period, nematodes completed 2-3 generations. Then IJs were recovered by White trap method (White, 1927). In this technique, a watch glass was kept upside down in the base of glass Petri plate. A moistened filter paper cut to size was kept on watch glass. Cadavers were transferred on it and covered by lid. Petri plates were sealed by parafilm to avoid contamination and loss of moisture. The Petri plates were enclosed in polythene bags and kept in a BOD incubator maintained at 25±1° C till the emergence of IJs. The IJs were collected in clean beaker by pouring water in Petri plate with wash bottle and stored in tissue culture flasks at 15±1° C in BOD incubator till used.

3.2 Natural occurrence of EPN fauna

3.2.1 Comparison the EPN fauna of disturbed and undisturbed ecosystems

Thirty samples, each from forest plantation (10 each from neem, eucalyptus and *sheesham*), fruit trees (jujube, guava and citrus) and vegetable crops (okra, onion and tomato) at CCS HAU, Hisar were collected from the rhizosphere at a depth of 5-10 cm with the help of *khurpi* in a polythene bag and properly labelled. Sampling spots were tagged with aluminium label and a rough map of sampling area was prepared to relocate these spots easily. These samples were brought to the laboratory. Soil was filled in the glass bottles. Five *G. mellonella* larvae per bottle were used as bait for isolation of EPNs (Plate 3). The insect mortality was checked daily till 7 days. Cadavers found, if any, were washed in distilled water to remove dirt particles and kept in Petri plate on dry filter paper fitted in lid. These Petri plates were kept inverted in a polythene bag and shifted to a BOD incubator maintained at 25±1° C for 8-12 days for *Heterorhabditis* spp. and 5 days for *Steinernema* spp. on the basis of colour acquired by the cadavers. The IJs were recovered by White trap method. IJs were collected in a beaker, mass multiplied and stored in tissue culture flasks at 15±1° C in a BOD incubator.

3.2.2 Seasonal occurrence of EPNs at selected sites

Three sites found positive for EPNs one each from neem, jujube and okra were selected for studying the occurrence of EPNs throughout the year. Monthly sampling was carried out at selected sites at fixed date and time to assess seasonal occurrence of EPNs. For sampling, three samples were collected from each site in polythene bag. Samples were brought in the lab and mixed properly. Ten ml water per sample was added if samples were too dry. Then samples were filled in empty glass bottles up to 2/3 rd of its capacity and five last instar *Galleria* larvae per bottle were used to isolate EPNs. The mortality of *Galleria* larvae was checked daily till 7 days. Infected cadavers were removed from bottles, washed in distilled water and kept on dry filter paper fitted in lid of Petri plate. Petri plates were kept inverted in a polythene bag and shifted to a BOD incubator maintained at 25±1° C. After 5-6

days of incubation in a BOD, infected cadavers were kept on White trap to harvest IJs. Soil temperature was recorded with the help of soil thermometer. Soil moisture was assessed by gravimetric method. Also soil texture was analyzed by International pipette method (Day, 1965) and general analysis of soil was done in collaboration with Department of Soil Science, CCS HAU, Hisar.

The data were analyzed statistically and correlation coefficient and regression equation were calculated using OPSTAT software available on CCS HAU website (www.hau.ernet.in).

3.3 Effect of cold stress on development and infectivity of EPNs inside host cadavers

This experiment was carried out in two sets.

3.3.1 Set - 1: Cold stress application at adult stage

G. mellonella larvae were infected with *Heterorhabditis indica* and *Heterorhabditis* (HP isolate) in sterile Petri plates by filter paper method separately and kept at 25±1° C in a BOD incubator. The dead insects were kept at 25±1° C till EPN species reaches the adult stage of 1st generation. To ensure this, 1-2 cadavers were dissected randomly and observed under stereozoom microscope. Then after attaining the required stage by the EPN, 10 plates per temperature containing 10 larvae per plate were shifted to temperatures of 5, 10, 15, 20±1° C. Control was maintained at 25±1° C. After setting the experiment, 6 cadavers per temperature per strain of EPN were dissected after every 4 days interval and developmental stage of EPN inside the cadaver was recorded. Such observations were recorded till appearance of IJs or maximum 28 days whichever was earlier. After fulfilling any one of these conditions, IJs were recovered by White trap method. Number of IJs emerging per temperature per plate was counted separately. Then recovered IJs were transferred on to modified Baermann funnel for collecting live and active nematodes. Infectivity of emerged IJs from each temperature and strain of EPN was tested by reinoculating the IJs to *Galleria* larvae @ 20 IJs per larva. Ten larvae per plate and 10 plates per temperature were used and infectivity was estimated by number of larvae killed after 3 days.

3.3.2 Set - 2: Cold stress application at juvenile stage

In this set, *Galleria* larvae were infected as in set 1. Cadavers were placed at 25±1° C till the 1st generation juveniles appeared and then shifted to lower temperatures as in set 1. IJs were recovered by White trap method after 20 days from each temperature. The emergence of IJs was recorded. Also, the infectivity was tested as in set 1.

The analysis of variance was carried out by completely randomized design using OPSTAT software (www.hau.ernet.in).

Plate 3: Insect Bait method for extraction of EPNs from soil

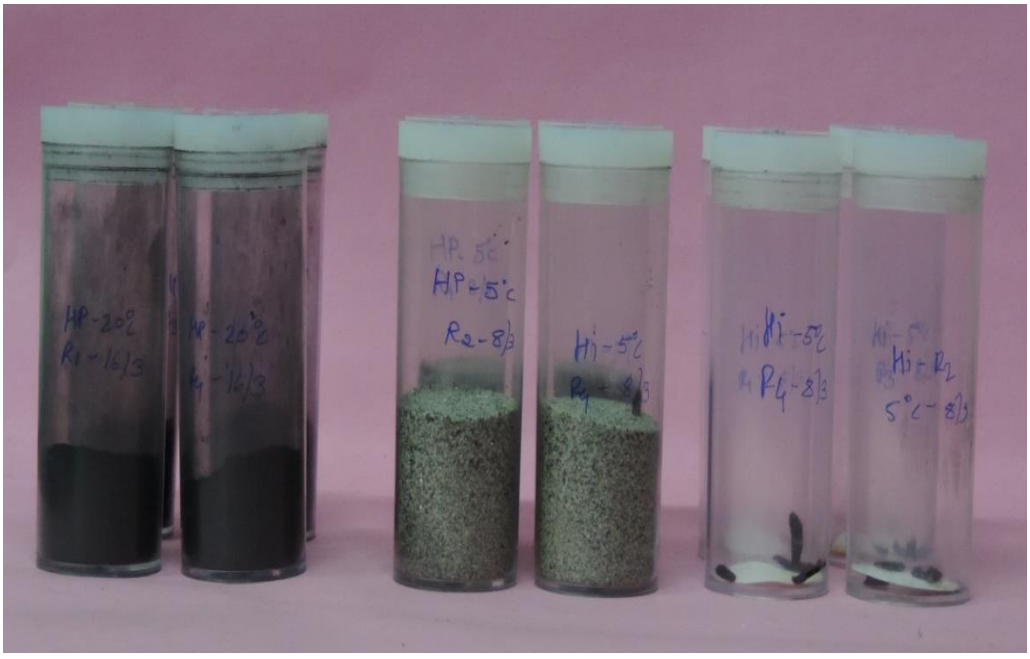

Plate 4: Media used for storage of cadavers: left - Charcoal powder; middle – River sand; right – Without medium, empty tubes lined with filter paper

3.4 Effect of temperature on EPNs inside host cadavers stored in inert media

In this experiment, three different media were used viz., sterilized river sand, activated charcoal powder and empty tubes with filter paper at bottom (Plate 4). River sand was sterilized in autoclave at 1.05 kg per sq cm and 121° C for 20 min; air dried and kept in 1000 ml conical flask plugged with non absorbent cotton. Activated charcoal was used for the purpose. Properly cleaned tubes (37 x 50 mm) were used as container. Twelve tubes per temperature per strain of EPN were filled; 4 each for sterilized river sand, sterilized charcoal powder and empty tubes with filter paper at bottom.

Galleria larvae were inoculated with *H. indica* and *Heterorhabditis* (HP Isolate) separately and allowed to develop up to IJs of second generation in a BOD incubator at 25±1° C. Then infected cadavers were surface-sterilized by putting cadavers in NaOCl for 30 sec to 1 min. Cadavers were thoroughly washed with distilled water for 2-3 times to remove traces of chemical. These wet cadavers were air dried in shade on filter paper for about 30 min.

Fifteen cadavers were kept per tube and care was taken that cadavers were fully immersed in respective media. Then tubes were closed with lid and shifted to respective temperature treatments (5, 10, 15, 20 and 25±1° C). Observations were taken after 15, 30, 60, 90 and 120 days on the emergence of IJs by removing three cadavers per tube and shifting it on White trap. Emerged IJs were collected in a beaker and counted by dilution method. In case there was no emergence, the cadavers were dissected and IJs were collected and counted.

The data were analyzed statistically by completely randomized factorial design (OPSTAT available on www.hau.ernet.in).

3.5 Compatibility of EPNs with insecticides

For this experiment the following six insecticides were used in recommended concentrations.

Sr.No.	Insecticide	Trade name	Concentration (%)
1	Imidacloprid	Confidor 17.8 SL	0.004
2	Novaluron	Rimon 10 EC	0.01
3	Spinosad	Tracer 45 SC	0.01
4	Thiodicarb	Larvin 75 WP	0.075
5	Triazophos	Hostathion 40 EC	0.08
6	Tap water (Control)		

A double strength solution of enlisted insecticides was prepared. IJs suspension of *H. indica* was standardized in such a way that one ml contained approximately 100 IJs. The experiment was laid in six well tissue culture plates (one plate for one pesticide treatment). One ml of the double strength solution was added per cavity along with 1ml of respective EPN (100 IJs) species in water. This assembly was kept at room temperature. The

observations were taken after 6, 12 and 24 h. While taking observations, the suspension in each cavity was stirred properly and 0.1 ml of suspension was poured in counting dish and observations on live and dead IJs were recorded. To confirm the dead IJs, they were touched with pick. If there was no movement, it was considered as dead.

The infectivity of these IJs was tested on *Galleria* larvae after completing the experiment. After 24 h, IJs were washed in water on 500 mesh sieve and recollected in a beaker. Again 6-well tissue culture plates, one per insecticide treatment were taken. A single layered filter paper was kept at bottom in each cavity and 0.1 ml of IJs suspension (40 IJs per 0.1 ml) was added. Two *Galleria* larvae per cavity were kept and observations on infectivity were recorded by observing mortality of insects. Only those insects with brick red colour were considered as killed by nematodes and per cent mortality was calculated.

3.6 Statistical analysis

Statistical analysis of the data was done using OPSTAT software (www.hau.ernet.in).

CHAPTER-IV

RESULTS

The results of the experiments on the natural occurrence of EPNs in disturbed and undisturbed ecosystems, effect of cold stress on the development, emergence and infectivity of EPNs, storage of EPNs inside the host cadaver in different media, and compatibility of IJs with different recommended insecticides, are presented in this chapter.

4.1. Experiment 1: To compare the EPN fauna of disturbed and undisturbed ecosystems

In total, 90 soil samples (10 samples per location) were collected from nine sites; three each from forest plantations (neem, eucalyptus and *sheesham*), fruit trees (jujube, guava and citrus) and vegetable crops (okra, onion and tomato). Maximum occurrence of EPNs was found at jujube (40%) and okra (40%) sites, followed by neem (30%). At both *sheesham* and tomato sites, only one (10%) site was found positive. No occurrence of EPNs was detected at citrus, guava, onion and eucalyptus sites (Table 1). Out of total 90, 13 sites were found positive with EPNs (12 *Steinernema* sp. and 1 *Heterorhabditis* sp.); thus it is inferred that *Steinerenema* is more prevalent than *Heterorhabditis*.

Based on the juvenile length, presence or absence of head papillae and colour of cadaver, the EPN isolates were assigned to different groups i.e., *Steinernema pakistanense* group (600-700 µm with papillae), *Steinernema abbasi* group (500-600 µm with papillae), *Steinernema asiaticum* group (< 500 µm without papillae), and *Steinernema bicornutum* group (> 700 µm, lateral lines = 9 and head papillae). Each sample contained only one species. All the populations are being identified separately.

Based on the occurrence of EPNs in various ecosystems, three sites - one representing each ecosystem, was selected for studying the natural persistence of the nematodes throughout the year (May, 2012 to April, 2013). Soil texture, pH and organic carbon were estimated by standard methods for the three sites (Table 2). Soil temperature and soil moisture were recorded at each sampling.

Soil texture and pH were not much variable at the three sampling sites. It was loamy sand with pH varying between 7.2 - 7.5. However, organic carbon was lower (0.60%) at vegetable field compared to jujube and neem locations (0.09%). The intensity of cropping, frequent irrigation and relatively disturbed ecosystem may be the contributing factors for lower organic carbon content at the vegetable field.

Table 1: Comparison of the EPN fauna in disturbed and undisturbed ecosystems

Location/ Plant	Number of samples collected	Number of samples positive with EPN	Frequency of occurrence (%)	EPN genus/group/species	No. of isolates
I. Forest plantation					
Neem	10	3	30	*Steinernema* sp. (*abbasi* group) *Heterorhabditis* sp.	Two
Eucalyptus	10	0	0	-	-
Sheesham	10	1	10	*Steinernema* sp. (*asiaticum* group)	One
II. Fruit trees					
Jujube	10	4	40	*Steinernema pakistanense* *Steinernema* sp. (*abbasi* group) *Steinernema* sp. (*asiaticum* group)	Two One One
Citrus	10	0	0	-	-
Guava	10	0	0	-	-
III. Vegetable crops					
Okra	10	4	40	*Steinernema* sp. (*abbasi* group) *Steinernema* sp. (*asiaticum* group)	Three One
Tomato	10	1	10	*Steinernema* sp. (*bicornutum* group)	-
Onion	10	0	0	-	-

Site I – Neem (Completely undisturbed ecosystem): *Steinernema* was recovered throughout the year (17.8-29.6° C), except in the months of January and February (15.1° C) (Table 2). A very highly significant ($p \leq 0.01$) correlation ($R^2 = 0.620$) was established between soil temperature and per cent larval mortality (Table 3). Soil moisture varied from 7.41 to 18.2 per cent and does not seem to affect the occurrence of EPNs significantly ($R^2 = 0.023$; $p \leq 0.05$) (Tables 2, 3; Fig. 1).

Site II – Jujube (Partially disturbed ecosystem): *S. pakistanense* was isolated in all the months (temperature > 17° C), except during November (26.5° C), December (17° C) and January (14.3° C). While in December and January low temperature could be the reason for non-detection of *S. pakistanense*, the same does not hold true for November (Table 2). At this site also, a very highly significant ($p \leq 0.01$) correlation ($R^2 = 0.513$) was established between soil temperature and per cent larval mortality (Table 3). Soil moisture continued to be less than 9 per cent from September to November but did not play any significant role ($R^2 = 0.089$; $p \leq 0.05$) (Table 3).

Site III – Okra (Disturbed ecosystem): The field was planted with okra – cabbage – *dhaincha* in that sequence. Being a vegetable field, the site was irrigated regularly, so soil moisture remained more than 9 per cent throughout. The site was fallow only for very short periods in between the crops. *Steinernema* sp. (*abbasi* group) was intercepted on all the sampling dates except during January (12.1° C) and February (15° C) (Table 2). Thus, it is inferred that low temperature may be responsible for non-detection of EPNs during winter months. A significant correlation ($R^2 = 0.388$; $p \leq 0.05$) was recorded between soil temperature and larval mortality. The role of soil moisture was non-significant ($R^2 = 0.013$; $p \leq 0.05$) (Table 3).

Table 2: Seasonal occurrence of EPNs at three sites

Month	Presence/Absence of EPN	Soil moisture (%)	Soil temperature (°C)	Larval mortality (%)
Site I -Neem(Loamy sand, pH 7.4, Organic Carbon = 0.90)				
May	√	11.03	26.3	100
June	√	08.67	27.6	100
July	√	15.59	27.5	80
August	√	06.90	29.6	80
September	√	18.19	29.6	100
October	√	10.19	27.1	60
November	√	07.41	26.3	100
December	√	09.42	17.8	40
January	X	09.25	15.1	0
February	X	11.91	15.1	0
March	√	11.96	19.8	100
April	√	11.86	25.1	100
Site II – Jujube(Loamy sand, pH 7.2, Organic Carbon = 0.90)				
May	√	08.87	30.5	100
June	√	05.72	32.0	100
July	√	21.45	31.5	100
August	√	15.24	29.1	100
September	√	08.83	31.3	100
October	√	05.75	27.5	100
November	X	06.10	26.5	0
December	X	20.92	17.0	0
January	X	15.65	14.3	0
February	√	11.29	17.1	60
March	√	10.13	22.6	100
April	√	07.38	26.5	100
Site III – Okra (Loamy sand, pH 7.5, Organic Carbon = 0.60)				
May	√	21.10	28.1	100
June	√	13.92	29.3	80
July	√	18.35	31.5	20
August	√	16.99	30.3	40
September	√	12.21	32.5	60
October	√	09.43	31.3	100
November	√	14.71	25.5	80
December	√	12.73	16.6	40
January	X	14.76	12.1	0
February	X	10.20	15.0	0
March	√	12.08	21.5	60
April	√	15.45	28.6	100

Table 3: Correlation between larval mortality, soil temperature and soil moisture at three field sites

Variable	Mean	Coefficient of Determination (R^2)	Regression Y = a + bx
Neem			
Y = Larval mortality	71.667	-	-
Soil temperature (°C)	23.959	0.620**	- 62.47+5.59x
Soil moisture (%)	11.036	0.023^{NS}	51.54+1.82x
Jujube			
Y = Larval mortality	71.667	-	-
Soil temperature (°C)	25.514	0.513**	- 58.82+5.11x
Soil moisture (%)	11.449	0.089^{NS}	98.90 – 2.37x
Okra			
Y = Larval mortality	56.667	-	-
Soil temperature (°C)	25.223	0.388*	- 25.20+3.24x
Soil moisture (%)	14.328	0.013^{NS}	38.33+1.28x

Y = Larval mortality (%); X = Variable factor; *Significant ($P \leq 0.05$); **Highly significant ($P \leq 0.01$)

4.2. Experiment 2: To study the effect of low temperature on development and infectivity of EPNs inside host cadavers

This experiment was carried out in two sets. In the first set the cold stress was given to adult nematodes, while in second set it was given to first generation juveniles and its effect on nematode development inside the host cadaver was observed.

Set I: The host cadavers infected with either *Heterorhabditis indica* or *Heterorhabditis* sp. (HP isolate) were allowed to develop up to 1st generation adults and then shifted to lower temperatures i.e., 20, 15, 10 and 5±1° C. Host cadavers maintained at 25±1° C served as control. Exposing *H. indica* at 1st generation adult stage to lower temperatures (20, 15, 10° C) did not hamper the development of the nematode to IJs of next generation and the life cycle was completed in 8 days; but at 5° C the development was stopped at adult stage itself (Table 4). Even after 28 days the dead females were recovered which were reddish brown in colour (Plate 6).

The number of IJs emerged was highest (414954) at 20° C, followed by 10 and 25° C. The least emergence (39810) was at 15° C. Infectivity of the emerged IJs was significantly less (92%) at 15 and 10° C compared to 25° C (control - 99%) and at 20° C (100%). The difference in infectivity in control (25° C) and 20° C was non-significant. At 5° C, there was no emergence, hence no infectivity (Table 6).

In *Heterorhabditis* sp. (HP isolate), development from adult to next generation IJs was completed in 8 days at all the temperatures, except 5° C; however, the emergence of IJs was delayed at 25° C by 4 days, while at 5° C IJs appeared only after 28 days which were mostly inactive (noodle-like appearance; Plate 7) and still inside the cuticle of the dead female (Table 5). However, these became active when allowed to pass through Baermann funnel assembly. These IJs were infective, though significantly less than that at 20 and 25° C.

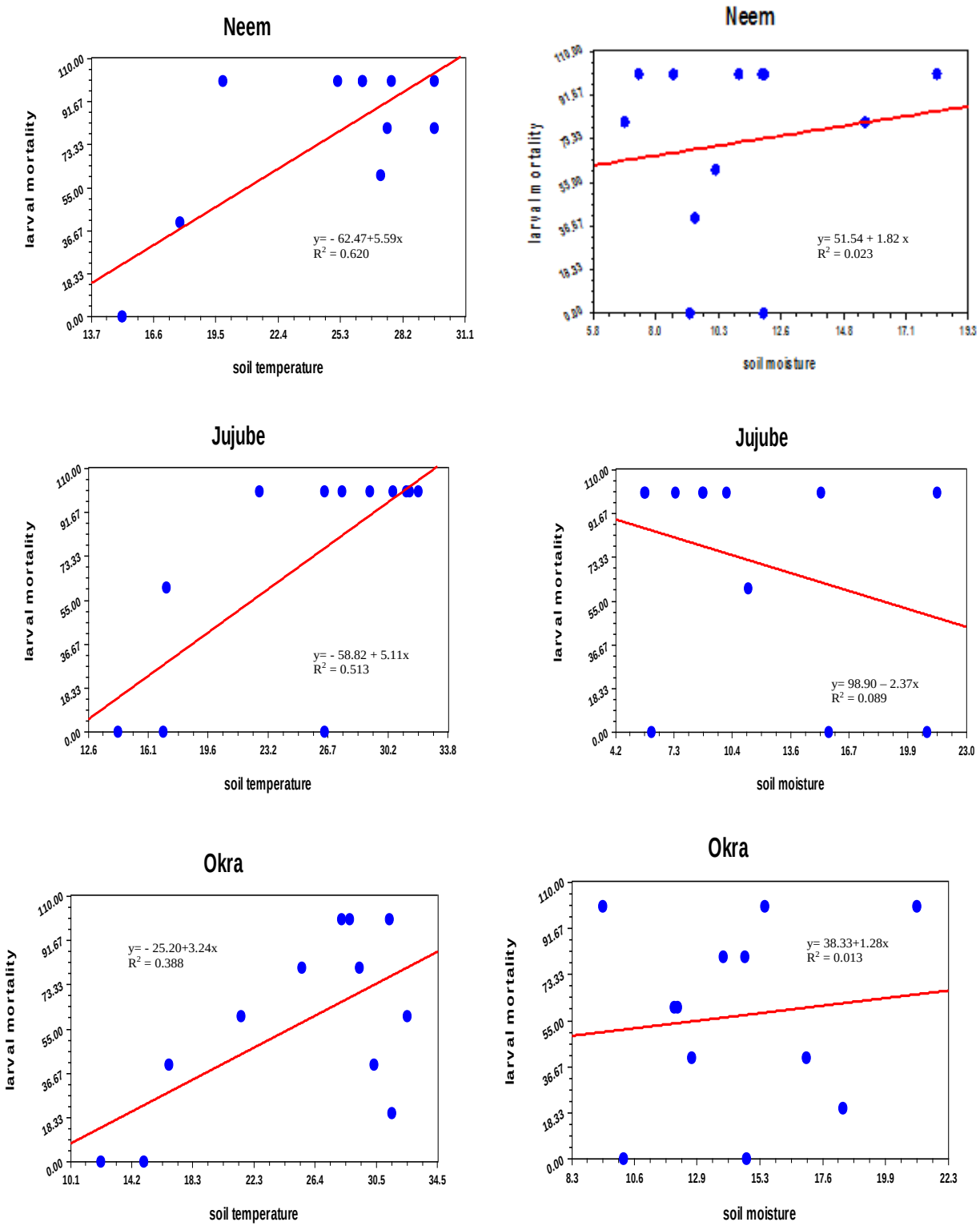

Fig. 1: Correlation between larval mortality, soil temperature and soil moisture at three field sites

Table 4: Effect of cold stress at adult stage on development of *Heterorhabditis indica* inside host cadavers

Storage Temp. (° C)	Storage period (days)						
	4	**8**	**12**	**16**	**20**	**24**	**28**
25 (Control)	5-10 1st generation females and few juveniles found; all active	Few 1st generation females, 100s of 2nd generation females and few juveniles found; all active	Few 1st generation females and 100s of 2nd generation females and juveniles found; all active	Thousands of IJs found in dissected *Galleria* larvae; life cycle completed	-	-	-
20	10-30 nematodes found - few juveniles, full grown and developing females	Few 1st generation females, 100's of 2nd generation females and few juveniles found, all active	Both 1st and 2nd generation females and 1000s of juveniles found; life cycle completed	-	-	-	-
15	10-16 1st generation females, 100s of 2nd generation females and juveniles found	Hundreds of 2nd generation females and juveniles found with very few 2nd generation females full of juveniles	2nd generation females and 100s of juveniles found	Life cycle completed but very few juveniles emerged	-	-	-
10	11-15 1st generation females, 100s of 2nd generation females and juveniles found	Thousands of juveniles found, all active; life cycle completed	-	-	-	-	-
5	Reddish coloured 1st generation dead females found	Reddish coloured 1st generation dead females found	Reddish coloured 1st generation dead females found	Reddish coloured 1st generation dead females found	Reddish coloured 1st generation dead females found	Reddish coloured 1st generation dead females found	Reddish coloured 1st generation dead females found

Table 5: Effect of cold stress at adult stage on development of *Heterorhabditis* sp. (HP isolate) inside host cadavers

Storage Temp.(° C)	Storage period (days)						
	4	**8**	**12**	**16**	**20**	**24**	**28**
25 (Control)	10-15 1st generation, few 2nd generation females and juveniles found	Few 1st generation and 100s of 2nd generation females found	1st and 2nd generation females and 1000s of active juveniles found	1000s of juveniles found in dissected larvae	-	-	-
20	1st generation females; few developed and few underdeveloped females found	1st and 2nd generation females, and 1000s of juveniles found; life cycle completed	-	-	-	-	-
15	1st generation females and 100s of juveniles found, all active	Thousands of IJs found; life cycle completed	-	-	-	-	-
10	1st generation females and few juveniles found, both active	Thousands of IJs found; life cycle completed	-	-	-	-	-
5	1st generation females live, less active; few IJs found, mostly inactive	All 1st generation females with inactive IJs inside; IJs found outside females mostly inactive	1st generation females with inactive IJs inside and outside in water; no body fluid oozed out upon puncturing	1st generation females and few IJs found; very few IJs active	1st generation females and 100s of IJs found, mostly inactive; colour of female changed to brownish	1st generation females and 100s of IJs found, mostly inactive; colour of female changed to brownish	Few 1st and 2nd generation females with 1000s of juveniles, most juveniles inactive; life cycle completed

Plate 5: *Heterorhabditis indica* females showing change in colour due to cold stress

Plate 6: *Heterorhabditis* sp. (HP isolate) infective juveniles showing "Noodle appearance" due to cold stress

Set II: Emergence of IJs was recorded after 20 days of laying the experiment. In *H. indica*, number of IJs emerged was significantly reduced at temperatures below 25° C, so much so that it was nil at 5° C. However, infectivity was significantly reduced in IJs emerging from cadavers maintained at 10° C (Table 7).

In *Heterorhabditis* sp. (HP isolate) IJ emergence was more from cadavers at 15 and 10° C when compared to 25 and 20° C, but infectivity was significantly reduced in these IJs. Unlike exposure of 1st generation adults to lower temperatures, exposure of 1st generation IJs to 5° C was detrimental since there was no emergence of IJs from such cadavers (Table 7).

In *H. indica*, exposure of 1st generation adults and juveniles to 5° C was detrimental for further development; whereas in *Heterorhabditis* sp. (HP isolate), adults tolerated the low temperature, but juveniles succumbed to the cold temperature.

Table 6: Effect of cold stress at adult stage on emergence and infectivity of *Heterorhabditis indica* and *Heterorhabditis* sp. (HP isolate) inside host cadavers (Set 1)

(mean of 8 replicates for emergence, 10 for infectivity)

Storage Temp. (° C)	*Heterorhabditis indica*		*Heterorhabditis* sp. (HP isolate)	
	Number of IJs emerged *	Infectivity (% mortality)**	Number of IJs emerged *	Infectivity (% mortality)**
25 (Control)	160013 (5.204)	99.00 (88.15)	65614 (4.816)	96.00 (82.61)
20	415089 (5.618)	100.00 (90.00)	53765 (4.729)	100.00 (90.00)
15	40500 (4.600)	92.00 (79.36)	45359 (4.656)	86.00 (71.38)
10	229725 (5.343)	92.00 (79.36)	61998 (4.783)	80.00 (64.80)
5	2.0 (0.3010)	00.00 (00.00)	13141 (4.097)	84.00 (68.72)
SE(m) ±	(0.027)	(2.868)	(0.029)	(3.085)
C.D. (P = 0.05)	(0.077)	(8.195)	(0.083)	(8.816)

Table 7: Effect of cold stress at juvenile stage on emergence and infectivity of *Heterorhabditis indica and Heterorhabditis* sp. (HP isolate) inside host cadavers (Set 2)

(mean of 10 replicates)

Storage Temp. (°C)	*Heterorhabditis indica*		*Heterorhabditis* sp. (HP isolate)	
	Number of IJs emerged*	Infectivity (% mortality)**	Number of IJs emerged*	Infectivity (% mortality)**
25 (Control)	357240 (5.539)	95.00 (81.80)	71257 (4.851)	100.00 (90.00)
20	214045 (5.331)	100.00 (90.00)	36100 (4.552)	100.00 (90.00)
15	2299 (3.305)	96.00 (84.68)	120406 (5.056)	92.00 (79.36)
10	201187 (5.323)	76.00 (63.66)	167075 (5.204)	92.00 (79.36)
5	2.0 (0.3010)	00.00 (00.00)	2.0 (0.3010)	00.00 (00.00)
SE(m) ±	(0.028)	(3.071)	(0.015)	(2.746)
C.D. (P = 0.05)	(0.079)	(8.776)	(0.043)	(7.848)

* Figures in parentheses are mean of log (n+2) transformation;

** Figures in parentheses are angular transformed values.

4.3 Experiment 3: To assess the storage of EPNs inside host cadavers

In this experiment, an effort was made to assess the feasibility of storing IJs of EPNs inside host cadavers by storing them in sterilized river sand or charcoal powder. Specimen tubes without medium but with a piece of filter paper at the base were used for comparison.

Both the EPN species could not be stored beyond 15 days at 25° C as the IJs emerged out in the tubes (Table 8, 9). At 20° C, both the species could be stored up to 30 days in sand as well as in tubes without medium, but not in charcoal powder in which cadavers became too dry and there was no emergence beyond 15 days. Maximum period for which either of these species could be stored was 90 days. At 15° C, *H. indica* could be stored up to 90 days whereas *Heterorhabditis* sp. (HP isolate) up to 60 days only in both sand and empty tubes. At 10° C, *H. indica* could be stored up to 90 days only in sand and the number of IJs emerging out was also reduced, whereas the other species could be stored in empty tubes as well and emergence of IJs was recorded though less than sand medium. *Heterorhabditis* sp. (HP isolate) could be stored for a month at 5° C too. Again charcoal was not a good medium for storage.

Table 8: Effect of temperature on *Heterorhabditis indica* inside host cadavers stored in inert media

(mean of 4 replicates)

Days	Number of IJs (log values) emerged in different media			Mean (Days)
	Empty	Sand	Charcoal	
25° C				
15	190542 (5.276)	156998 (5.194)	6351 (3.157)	
20° C				
15	399819 (5.587)	155277 (5.184)	2.0 (0.3010)	(3.590)
30	121465 (5.079)	338444 (5.503)	2.0 (0.3010)	(3.527)
Mean (Medium)	(5.333)	(5.343)	2.0 (0.3010)	
SE(m) ± CD (P = 0.05)	Days (0.0302) (N.S.)	Media (0.0370) (0.1099)	Days x Media (0.0523) (0.1550)	
15° C				
15	336111 (5.485)	31250 (4.401)	131111 (5.118)	(5.334)
30	275754 (5.389)	32258 (4.494)	2.0 (0.3010)	(3.294)
60	72333 (4.839)	25111 (4.319)	2.0 (0.3010)	(3.053)
90	6666 (3.816)	3777 (3.566)	2.0 (0.3010)	(2.460)
180	2.0 (0.3010)	2.0 (0.3010)	2.0 (0.3010)	(0.3010)
Mean (Medium)	(3.906)	(3.556)	(1.024)	
SE(m) ± CD (P = 0.05)	Days (0.0509) (0.1469)	Media (0.0394) (0.1138)	Days x Media (0.0881) (0.2550)	
10° C				
15	50699 (4.705)	42756 (4.631)	2.0 (0.3010)	(3.112)
30	7328 (3.865)	30690 (4.487)	2.0 (0.3010)	(2.784)
60	10 (1.006)	8 (0.905)	2.0 (0.3010)	(0.637)
90	2.0 (0.3010)	46 (1.665)	2.0 (0.3010)	(0.555)
Mean (Medium)	(2.394)	(2.922)	(0.3010)	
SE(m) ± CD (P = 0.05)	Days (0.279) (0.800)	Media (0.242) (0.693)	Days x Media (0.483) (1.386)	
5 ° C				
15	-	-	-	

Figures in parentheses are mean of log (n+2) transformation

Table 9: Effect of temperature on *Heterorhabditis* sp. (HP isolate) inside host cadavers stored in inert media

(mean of 4 replicates)

Days	Number of IJs (log values) emerged in different media			Mean (Days)
	Empty	Sand	Charcoal	
25° C				
15	171666 (5.229)	161383 (5.145)	2.0 (0.3010)	
20° C				
15	297333 (5.456)	151555 (5.160)	3291 (3.641)	(4.753)
30	115000 (5.052)	46458 (4.658)	2.0 (0.3010)	(3.237)
Mean (Medium)	(5.254)	(4.909)	(1.821)	
SE(m) ± CD (P = 0.05)	Days (0.030) (0.088)	Media (0.036) (0.108)	Days x Media (0.051) (0.154)	
15° C				
15	198333 (5.494)	334375 (5.511)	2.0 (0.3010)	(3.668)
30	231172 (5.270)	294591 (5.229)	2.0 (0.3010)	(3.500)
60	127574 (5.038)	206287 (5.158)	2.0 (0.3010)	(3.399)
Mean (Medium)	(5.267)	(5.299)	(0.3010)	
SE(m) ± CD (P = 0.05)	Days (0.065) (0.189)	Media (0.065) (0.189)	Days x Media (0.112) (NS)	
10° C				
15	149444 (5.128)	103333 (5.011)	2.0 (0.3010)	(3.380)
30	62555 (4.790)	38250 (4.512)	2.0 (0.3010)	(3.100)
60	58000 (4.701)	19833 (4.205)	2.0 (0.3010)	(2.969)
90	1050 (3.014)	1963 (3.290)	2.0 (0.3010)	(2.101)
Mean (Medium)	(4.408)	(4.254)	2.0 (0.3010)	
SE(m) ± CD (P = 0.05)	Days (0.049) (0.141)	Media (0.043) (0.122)	Days x Media (0.085) (0.245)	
5° C				
15	15761 (4.182)	13083 (4.111)	2.0 (0.3010)	(2.764)
30	15159 (4.179)	10791 (4.0320	2.0 (0.3010)	(2.737)
Mean (Medium)	(4.181)	(4.071)	(0.3010)	
SE(m) ± CD (P = 0.05)	Days (0.019) (N.S.)	Media (0.024) (0.071)	Days x Media (0.034) (NS)	

Figures in parentheses are mean of log (n+2) transformation

4.4 Experiment No 4: To ascertain the compatibility of EPNs with selected insecticides for field applications

A few common insecticides were tested at recommended doses by water screening method to ascertain their compatibility with EPNs for field application. The data were analyzed by two factor completely randomized design (Table 10). The per cent survival of IJs ranged from 98.1 – 100 in insecticidal concentrations which were at par with control (99.4%). Taking exposure time into consideration, the survival was not decreased even after 24 h of exposure – the time sufficient for the IJs to infect an insect host. The IJs were tested further for infectivity on *Galleria* larvae and were found infective in all the treatments.

Table 10: Effect of insecticides on survival of *Heterorhabditis indica* IJs

(mean of 6 replicates)

Treatments	Concentration (%)	Per cent survival of IJs			Means (Concentration)
		6 h	12 h	24 h	
Triazophos	0.08	100.00 (85.91)	98.80 (84.00)	96.48 (80.94)	98.42 (83.61)
Novaluron	0.01	100.00 (85.911)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)
Spinosad	0.01	100.00 (85.911)	98.48 (83.66)	100.00 (85.91)	99.49 (85.16)
Thiodicarb	0.075	98.61 (83.79)	97.29 (81.75)	98.61 (83.79)	98.17 (83.11)
Imidacloprid	0.004	98.71 (83.90)	100.00 (85.91)	98.33 (83.51)	99.01 (84.44)
Control (water)		100.00 (85.91)	98.14 (83.34)	100.00 (85.91)	99.38 (85.05)
Means (Time)		**99.55 (85.22)**	**98.78 (84.09)**	**98.90 (84.33)**	

Values in parentheses are angular transformed

	Concentration	Time	Concentration x Time
SE (m) ±	(0.97)	(0.69)	(1.68)
C.D. (P = 0.05)	(N.S.)	(N.S.)	(N.S.)

CHAPTER-V

DISCUSSION

Soil dwelling entomopathogenic nematodes are found world over in different habitats, except Antarctica. They are efficient bio-control agents of insects pests and have generated great interest owing to their no-residue and eco-friendly approach for environment. Understanding diversity and distribution of EPNs in various ecosystems, effect of temperature on the development and infectivity of IJs and storage methods to increase shelf life of formulations, and compatibility with insecticides are important for selecting a species for use against insects in a particular ecosystem. The present investigations were carried out in the light of these objectives, and the results obtained are discussed in this chapter in the backdrop of similar work done earlier.

The EPNs were encountered at 13 out of total 90 sites investigated. These are equally frequent in arable and non-arable ecosystems. Any bio-control agent is successful if isolated indigenously from the site where it is to be used since it is well adapted to the prevailing environmental conditions since exotic species may have detrimental effect on non-target species (Bathon, 1996; Ehlers and Hokkanen, 1996; Lynch and Thomas, 2000; Millar and Barbercheck, 2001). Hence, efforts are being made by the scientists to isolate species/strains indigenous to their area and adapted to local environmental conditions to restrict the inoculative release of the organism. The present investigations were a step in this direction. EPNs have been reported from various parts of the world (Hominick *et al*., 1996; Hominick, 2002; Adams *et al*., 2006) and India as well (Hussaini, 2003).

Occurrence of nematodes in all the three types of ecosystems was attributed to soil type – loamy sand, soil temperature and may be availability of insect host. Similar studies in Spain revealed the presence of 20 isolates from 14 sites having varied habitat, soil type and other physico-chemical factors. *Steinernema feltiae* was most common species with wide range of altitude, temperature, rainfall, pH and soil moisture but preferred sandy soils (Campos-Herrara *et al*., 2007). The variation in the occurrence of species was noticed in three agro-ecological zones of Southern Cameroon. *Heterorhabditis baujardi* was frequent in forest and fruit crops whereas *Steinernema* sp. was found in nine tree plantations of teak and another species of the genus in a forest habitat. Nematodes were present in pH range of 3.7 to 7.0 and in loamy and clayey soils with sandy content but not in sand, loamy sand and clay loam soils

(Kanga *et al*., 2012). Our observations were contrary to these since *Steinernema* was more frequent than *Heterohrbditis* and soil type was loamy sand with a pH range of 7.2-7.6 at three selected sites.

The frequency of occurrence was 15.5 per cent in 90 samples in the present studies. Similar frequencies have been reported from the world over; 6.9 per cent in Ethiopia (Mekete *et al*., 2005); 7 per cent from Chile (Edgington *et al*., 2010), 20.04 per cent in south Andamans (Prasad *et al*., 2001a) and 52 per cent in Kenya (Mwaniki *et al*., 2008). Variations were attributed to sampling methods, use of bait insect and sampling sites (insect habitats or outbreaks). Absence of EPNs in citrus and guava plantation highlights the more intensive surveys since the distribution of EPNs too is patchy (Hominick, 2002). In eucalyptus trees and onion crop, non recovery of EPNs may be due to allelochemical effect of these crops in the soil. Though insect nematodes are often more frequently isolated in reduced tillage system (Stuart *et al*., 2006), in okra ecosystem was disturbed, but okra-cabbage-*dhaincha* rotation and frequent irrigation may have provided congenial environment for insects and hence EPNs were recorded in 40 per cent of the samples collected.

The pH at three sites was within the range (7.2-7.6) since optimal pH range 4-8 has been specified earlier by Kung *et al*. (1990). Also the loamy sand soil texture was suitable for EPN as Kung *et al*. (1990) showed the preference of EPN for lighter soil textures. Depending on the occurrence of these nematodes in different types of ecosystems, the species can be selected on the basis of crops.

EPNs do employ some survival strategies to overwinter the cold conditions so that they are able to infect when the temperature conditions improve. Steinernematids can survive freezing temperatures (Molyneux, 1984, 1986; Brown and Gaugler, 1996) and heterorhabditids show infectivity in the 6-12° C range (Griffin and Downes, 1991). It has been demonstrated that both temperature and inoculum size greatly influence the development time, the duration of emergence, and the yields of IJs of *S. carpocapsae* (Kaya, 1977). Similar observations were recorded by Zervos *et al*. (1991) for *S. glaseri* in *G. mellonella*.

Campbell *et al*. (1995) indicated that nematode populations do migrate vertically to overwinter adverse environmental conditions. Impacts of fluctuating temperatures on development and infectivity of *S. carpocapsae* was studied inside host cadavers and reported that population survival was reduced by 95 per cent at lower temperatures and emerging IJs from cold stressed hosts were not infectious (Bornstein-Forst *et al*., 2005).

In Haryana conditions, soil temperature during winters goes down to 12° C from 32° C in summer and how EPNs isolated from these areas survive during winters formed part of this study. An EPN species isolated from cold region of Himachal Pradesh (HP isolate) was included in this study for comparison. The host cadavers protect the IJs during freezing

(Lewis and Shapiro-Ilan, 2002). So, host cadavers infected with *H. indica* or *Heterorhabditis* (HP isolate) were used. Only 5° C was found detrimental for the development of *H. indica* both at adult and juvenile stages whereas *Heterorhabditis* (HP isolate) was able to complete its development when exposed at adult stage and not when exposed at juvenile stage. Emerged juveniles were infective at all temperatures. In *H. indica*, there was no infectivity at 5° C whereas IJs were infective in *Heterorhabditis* (HP isolate) at the same temperature.

In nature, EPNs are exposed to fluctuations in temperature and how development occurs in nature in such conditions was studied in these investigations. The arrest of development at 1st generation adult stage at 5° C suggests that exposure to low temperature conditions at early stage is detrimental and no IJs are produced in *H. indica* and the same is not true for *Heterorhabditis* (HP isolate) a cold adapted species. In the latter case IJs were produced though life cycle was delayed by 20 days and mostly inactive juveniles gave noodle-like appearance – a response to low temperature and moisture conditions; while at juvenile stage no emerging population was recovered.

Similar studies have earlier been carried out by Borstein-Forst *et al*., 2005. Quite unlike their studies, the IJs were infective in the present investigations. Host cadaver provided protection to *H. bacteriophora*, *S. carpocapsae, S. glaseri* and *S. feltiae* from freezing temperatures. Highest stage specific survival was IJs for *S. carpocapsae* and adults for *H. bacteriophora* as observed by Lewis and Shapiro-Ilan, 2002. Brown and Gaugler (1996) found that exposure of cadavers with EPNs to conditions of cold temperatures and low humidity delayed emergence, thereby enhancing IJ survival in desiccating conditions. KoppenhÖfer *et al*. (1997) too observed higher rate of IJ survival inside host cadaver during dry soil conditions.

A number of EPN formulations (gels, clays and water dispersible granules) have been researched by various workers with shelf life of the products ranging from a few weeks to up to 12 months as enlisted by Grewal and Peters, 2005. Small quantities of nematodes can easily be transported in vermiculite and polyether polyurethane sponge as inactive carriers. Since the nematodes are active in these formulations, constant refrigeration is required and shelf-life is short (3-4 months). Active carriers viz., flowable gels, alginate capsules etc. are used in some formulations to reduce nematode activity and metabolism through the induction of partial anhydrobiosis. This allows the storage of EPNs at room temperature (Georgis, 1990; Grewal, 2002). Use of hydrogenated oil paste in formulations further increases the shelf-life (Chang and Gehert, 1995). Inducing partial anhydrobiosis by controlling water activity of the substrate through the composition of formulation ingredients increases shelf-life (Bedding, 1988; Silver *et al*., 1995; Grewal, 2000 a, b). Host cadavers provide protection to the IJs and hence host cadaver-based formulations were developed by Shapiro-Ilan *et al*., 2001. How these host cadavers can be stored, was the third objective of this study. Charcoal powder and

sand were used as inert storage media. Empty tubes with a filter paper disc at the base served as control. Charcoal powder was found unsuitable for the storage medium since the IJs inside were dehydrated beyond recovery. *H. indica* (a warm temperature adapted species) cadavers could be stored maximum for 90 days both at 15 and 10° C in sand as well as in empty tubes though there was a loss in the number of IJs emerged with the increase in time of storage. At 5° C there was no emergence. In *Heterorhabditis* sp. (HP isolate) a cold adapted species, the cadavers could be stored for 60 days at 15° C, 90 days at 10° C and only 30 days at 5° C. The number of IJs emerged was not reduced with time in this species. In earlier studies it has been postulated that low temperature (2-5° C) reduces metabolic activity and enhances shelf-life but optimum temperature differs with nematode species. The species isolated from warmer areas viz., *H. indica, S. riobrave* cannot be stored below 10° C (Strauch *et al*., 2000; Grewal, 2002). Studies by Campbell *et al*. (1998) indicate that IJs overwinter the adverse temperature conditions by vertical migration. However, the IJs still inside the host cadaver are not able to migrate and hence their development, emergence and infectivity after the emergence and infectivity after the return of congenial condition are liable to be affected. At low temperature (14 and 10° C), IJs inside host cadavers failed to develop and all populations die (Bornstein-Forst *et al*., 2005). However, in the present study host cadaver provided protection up to 10° C in *H. indica* and up to 5° C in *Heterorhabditis* sp. (HP isolate) depicting the variation in behaviour of two species depending on the area of their origin i.e., warm or cold. The study further revealed that sand is a good medium for storage at 15° C. Further studies can be carried out using charcoal powder with sand for inducing partial anhydrobiosis in IJs to reduce their metabolic activity and increase shelf life.

In order to study the compatibility of insecticides at recommended doses with EPN species (*H. indica*) laboratory assays were conducted by water screening method. The per cent survival of IJs ranged between 98.1 to 100 in all insecticides. The exposure to insecticides was not detrimental even after 24 h. Most of the EPN species infect the insect host within 24 h and hence the survival in insecticides up to 24 h is enough to use them in IPM system against insect pests.

There are conflicting reports regarding the safety of organophosphates, carbamates and neem products to EPNs. While Kaya (1985) and Zimmerman and Cranshaw (1990) reported that EPNs and chemical pesticides are generally compatible. Rovesti and Deseco (1990) observed that neem seed kernel extract was more toxic to heterorhabditids than steinernematids. The difference in the results can be attributed to the use of different species of nematodes. The different species of nematodes react differently to the various insecticides. Gupta and Siddiqui (1999) reported that *S. carpocapsae* was generally compatible with endosulfan, phosphamidon, cypermethrin, malathion, monocrotophos, dithane M-45, copper oxychloride, agallol and 2, 4-D sodium salt. Baur *et al.* (1998) reported that the combination of *S. carpocapsae* + *Bacillus thuringiensis* (*Bt*) resulted in to 58 per cent control of *Plutella*

xylostella compared to only 41 per cent and 44 per cent by *S. carpocapsae* and *Bt* alone, respectively. KoppenhÖfer *et al.* (1999) obtained maximum control against scarab grubs with combination of nematode and *Bt*.

CHAPTER-VI

SUMMARY AND CONCLUSION

The results of the experiments on the natural occurrence of EPNs in disturbed and undisturbed ecosystems, effect of cold stress on the development, emergence and infectivity of EPNs, storage of EPNs inside the host cadaver in different media, and compatibility of IJs with different recommended insecticides, are summarised in this chapter.

Comparison the EPN fauna of disturbed and undisturbed ecosystems

- Ninety soil samples (10 samples per location) were collected from nine sites; three each from forest plantations (neem, eucalyptus and *Sheesham*), fruit trees (jujube, guava and citrus) and vegetable crops (okra, onion and tomato).
- Maximum occurrence of EPNs was found at jujube and okra sites (40% each), followed by neem (30%) while at *sheesham* and tomato sites, 10 samples were found positive. No EPNs were detected at citrus, guava, onion and eucalyptus sites.
- Out of total 90, 12 sites were found positive with *Steinernema* sp. and only one with *Heterorhabditis* sp
- Based on the juvenile length, presence or absence of head papillae and colour of cadaver, the EPN isolates were assigned to different groups i.e., *Steinernema pakistanense* group, *Steinerrnema abbasi* group, *Steinernema asiaticum* group, and *Steinernema bicornutum* group.
- On the basis of their occurrence, three sites - one representing each ecosystem was selected for studying the natural persistence of the nematodes throughout the year.
- Soil texture and pH were not much variable at the three sampling sites. It was loamy sand with pH varying between 7.2 - 7.5, however, organic carbon was lower at vegetable field compared to jujube and neem locations.
- At site I – Neem (completely undisturbed ecosystem), *Steinernema* was recovered throughout the year, except in the months of January and February. A highly significant correlation was established between soil temperature and per cent larval mortality of EPNs. Soil moisture did not affect the occurrence of EPNs significantly.
- At site II – Jujube (partially disturbed ecosystem), *S. pakistanense* was isolated in all the months, except during November, December and January. At this site also, a very highly significant correlation was established between soil temperature and per cent

larval mortality of EPN. Soil moisture continued to be less than 9 per cent from September to November but did not play any significant role.

- At site III – Okra (disturbed ecosystem), the field was planted with okra – cabbage – *dhaincha* sequence. *Steinernema* sp. (*abbasi* group) was intercepted on all the sampling dates except during January and February. A significant correlation was recorded between soil temperature and larval mortality. The role of soil moisture was non-significant.

Effect of low temperature on development and infectivity of EPNs inside host cadavers

- In the first set, the cold stress was given to adult nematodes, while in second set it was given to first generation juveniles.
- In the set 1, the host cadavers infected with either *Heterorhabditis indica* or *Heterorhabditis* sp. (HP isolate) were allowed to develop up to 1st generation adults and then shifted to lower temperatures i.e., 20, 15, 10 and 5±1° C.
- Exposing *H. indica* at 1st generation adult stage to lower temperatures (20, 15, 10° C) did not hamper the development of the nematode to IJs of next generation and the life cycle was completed in 8 days; but at 5° C the development was stopped at adult stage itself.
- The number of IJs emerged was highest at 20° C, followed by 10 and 25° C. The least emergence was at 15° C. Infectivity of the emerged IJs was significantly less at 15 and 10° C compared to 25° C (control) and at 20° C. The difference in infectivity in control and 20° C was non-significant. At 5° C, there was no emergence, hence no infectivity.
- In *Heterorhabditis* sp. (HP isolate), development from adult to next generation IJs was completed in 8 days at all the temperatures, except 5° C; however, the emergence of IJs was delayed at 25° C by 4 days, while at 5° C IJs appeared only after 28 days. These IJs were infective, though significantly less than that at 20 and 25° C.
- In set 2, in *H. indica*, number of IJs emerged was significantly reduced at temperatures below 25° C, and it was nil at 5° C. However, infectivity was significantly reduced in IJs emerging from cadavers maintained at 10° C.
- In *Heterorhabditis* sp. (HP isolate) IJ emergence was more from cadavers at 15 and 10° C when compared to 25 and 20° C, but infectivity was significantly reduced in these IJs.
- Unlike exposure of 1st generation adults to lower temperatures, exposure of 1st generation IJs to 5° C was detrimental.

- In *H. indica*, exposure of 1st generation adults and juveniles to 5° C was detrimental for further development; whereas in *Heterorhabditis* sp. (HP isolate), adults tolerated the low temperature, but juveniles succumbed to the cold temperature.

Assessment of the storage of EPNs inside host cadavers

- Both *H. indica* and *Heterorhabditis* (HP isolate) could not be stored beyond 15 days at 25° C.
- At 20° C, both the species could be stored up to 30 days in sand as well as in tubes without medium, but not in charcoal powder.
- Maximum period for which either of these species could be stored was 90 days.
- At 15° C, *H. indica* could be stored up to 90 days, whereas *Heterorhrabditis* (HP isolate) up to 60 days only in both sand and empty tubes.
- At 10° C, *H. indica* could be stored up to 90 days only in sand and the number of IJs emerging out was also reduced, whereas the other species could be stored in empty tubes as well and emergence of IJs was recorded though less than sand medium.
- *Heterorhabditis* sp. (HP isolate) could be stored for a month at 5° C too and charcoal was not a good medium for storage.

Compatibility of EPNs with selected insecticides for field applications

- Five commonly used insecticides namely, triazophos, novaluron, spinosad, thiodicarb and imidacloprid were tested at recommended doses for their compatibility with both *H. indica* and *Heterorhabditis* (HP isolate) in laboratory assays.
- The per cent survival of IJs ranged from 98.1-100 in insecticidal concentrations which were at par with control.
- The survival was not decreased even after 24 h of exposure, the time sufficient for the IJs to infect an insect host.

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ABSTRACT

Title of thesis	**:**	**Bio-ecology of Entomopathogenic Nematodes**
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Title of degree	**:**	M. Sc. (Nematology)
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Out of 90 samples collected from forest plantations (neem, eucalyptus and *sheesham*), fruit trees (jujube, guava and citrus) and vegetable crops (okra, onion and tomato), maximum occurrence of EPNs was found at jujube and okra sites (40% each), followed by neem (30%), while at *Dalbergia* sp. and tomato sites only10% sites were found positive. Twelve sites were found positive with *Steinernema* sp. and only one with *Heterorhabditis* sp. In surveillance studies, at site I – Neem (completely undisturbed ecosystem), site II – Jujube (partially disturbed ecosystem) and site III – Okra (disturbed ecosystem), *Steinernema* spp. were recovered throughout the year, except in January and February. A highly significant correlation was established between soil temperature and per cent larval mortality of EPNs. When cold stress was given to adult of *H. indica* and *Heterorhabditis* sp. (HP isolate) at 20, 15, 10° C, the development of the nematode to IJs of next generation was not hampered; but at 5° C the development was stopped at adult stage itself in *H. indica* but delayed in the other species. Cold stress given to 1st generation juveniles of *H. indica* reduced IJ emergence and infectivity; and it was nil at 5° C. In *Heterorhabditis* sp. (HP isolate) IJ emergence was more but infectivity was reduced at 15 and 10° C; whereas in *Heterorhabditis* sp. (HP isolate), adults tolerated the low temperature, but juveniles succumbed to the cold temperature. Both *H. indica* and *Heterorhabditis* (HP isolate) could not be stored beyond 15 days at 25° C. At 20° C, both the species could be stored up to 30 days in sand as well as in tubes without medium, but not in charcoal powder. Maximum period for which either of these species could be stored was 90 days at 10 or 15° C. *Heterorhabditis* sp. (HP isolate) could be stored for a month at 5° C. Triazophos, novaluron, Spinosad, thiodicarb and imidacloprid were compatibility with *H. indica*. The per cent survival of IJs ranged from 98.1-100. The survival was not decreased even after 24 h of exposure.

MAJOR ADVISOR **DEGREE HOLDER**

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