PARASITOLOGY
Taxonomy and Bioecology

Editor
Sushil Kumar Upadhyay, D.Phil (Sci.)
FISEC, FSESc, FSSc, FSILsc, FISCA, FZSI, FAGEM, FHSI, FIAZ, FMERC, FBPS, FSEZR
Fmr. SO, Wildlife Crime Control Bureau, Government of India
Assistant Professor, Department of Biotechnology
Maharishi Markandeshwar (Deemed to be University)
Mullana-Ambala, (Haryana), India

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Indiscriminate use of pesticides and insecticides has caused an alarming threat to both environment as well as human health. This has diverted the attention of scientists to find out natural enemies of insect pests. Entomopathogenic nematodes have displayed a satisfactory potential in this regard calming to get rid of insect pests through eco-friendly and highly specific approach. Ease in their mass production and high compatibility with other control agents has raised their importance in integrated pest management. Molecular insights related to symbiotic association between nematode and bacteria in their gut, specific markers, genomic and proteomic studies have further explored many dimensions which will serve the field in near future. These biocontrol agents have been accepted worldwide which is evident from huge literature availability related to them.

INTRODUCTION

Among nematodes, Entomopathogenic nematodes have emerged as reliable biocontrol agents displaying large host range, good virulence and safety of non target species. Their formulation stability, easy application and cost/benefit ratio has raised their competitive potential against chemical insecticides (Georgis, 1992). There are two most popular families i.e., Heterorhabditidae and Steinernematidae which are
extensively used for bio-control of insect pests. They possess symbiotic bacteria *i.e.* *Photorhabdus* spp. and *Xenorhabdus* spp. respectively in the gut of infective juveniles (IJJs). These IJJs enter into the haemocoel of host through external body openings or cuticle and release these bacteria there. The bacteria multiply in the haemolymph of the insect and kill it due to septicemia. Meanwhile, the nematode feed upon bacteria and reproduces to complete 2-3 generations. After the death of host, the IJJs leave it to find a new host (Goodrich-Blair and Clarke, 2007). But there are contrary reports also which state that “Activated Infective Juvenile” of *Steinernema carpocapsae*, release venom proteins which is lethal to various insects including *Drosophila melanaster*. It has been confirmed by studies that even in mutualism the EPN especially *S. carpocapsae* accounts for more Pathogenicity as compared to bacteria (Lu et al., 2017).

**NEMATODE CULTURE IN LABORATORY CONDITIONS**

Nematode cultures are maintained in the laboratories on *Galleria mellonella* larvae. It can be reared on artificial diet having following ingredients Wheat bran (100 g), Maize flour (200 g), Milk powder (100 g), Yeast (30 g), Honey (125 ml) and Glycerol (125 ml). Extraction of nematodes is done through baiting with *G. mellonella* larva and white trap method (White, 1929) (Fig. 3.1). The extracted nematodes are harvested daily and cleaned properly to obtain pure cultures. Identification of nematodes is done through morphometry and molecular characterization. Storage of pure nematodes is done in sterilized soil or in the form of cadavers.

![Fig 3.1. An outline of entomopathogenic nematode extraction.](image)
EPN OCCURRENCE AND THEIR INTERACTION WITH SOIL

Many studies have been conducted for survey of nematodes in different types of habitats. These have been reported from sudan grass fields (Molina-Ochoa et al., 2003), woodland and grassland (Torr et al., 2007), cultivated and non-cultivated areas (Kary et al., 2009), agronomic crops, pastures and forests (Barbosa-Negrisoli et al., 2010), citrus groves (Campos-Herrera et al., 2013), crop fields and forest soil, (Erba et al., 2014; Kaushik and Chaubey, 2014; Razia and Sivaramakrishnan 2014), Ginger rhizosphere (Pervez et al., 2014), oak forests, grasslands and alfalfa fields (Abdolmaleki et al., 2016), corn, grain, forage sorghum and many more.

Although the distribution of EPNs is cosmopolitan but studies have reported that their occurrence is uneven. The number of positive samples recovered from various surveys varies from place to place like 12.3% from Belgium (Miduturi et al., 1996), 10.5% from Republic of Ireland (Griffin et al., 1991), 10% from Japan (Yoshida et al., 1998), 5% from Italy (Ehlers et al., 1991), 2.2% from Scotland (Boag et al., 1992) etc. These reports indicate that EPNs show different preference for various habitats. Studies have reported that *S. silvaticum* preferred forest habitat (Sturhan, 1995) whereas *S. affine* and *S. feltiae* preferred arable, meadows, verges and roadside habitats (Mracek et al., 2005). Nematode occurrence and their distribution have also been related with the abundance of insect host, which create an ideal environment for the EPNs (Mracek et al., 2005). In an ecosystem, many interactions are going on between organic matter, soil minerals and microorganisms which ultimately affect its biological and physico-chemical properties. This makes it necessary to understand the physico-chemical characteristics of the soil with the nematode prevalence. Kung et al. (1990) reported decrease in survival as well as pathogenicity of Steinernematids with decrease in pH from 8 to 4 whereas; it decreased drastically at pH 10. This can be related to the abundance of infective juvenile stage which is highly pathogenic. Studies on soils of Assam, India have also linked sand, loam and high moisture with abundance of entomopathogenic nematodes (Bora et al., 2015). In the soils of Portugal, Valadas et al. (2014) also conducted similar studies but found no clear association between EPN species and the type of soil. In another study on the soils from abandoned agriculture terraces in North Lebanon, Nader et al. (2010) reported that EPNs presence is correlated with soil characteristics like humidity, porosity, texture and organic matter where as vegetation or soil pH showed no linkages with EPNs. Moisture plays a significant role in regulating abundance of the soil organisms (Kennedy, 1993). Establishment of nematode community has been linked with pH, EC, C/N ratio and soil moisture through studies on soils of Antarctica; the natural laboratories on planet earth (Courtright et al., 2000, 2001). Both positive as well as negative correlations of moisture with occurrence of nematodes are confirmed by many studies (Powers et al., 1998; Powers et al., 1998; Treonis et al., 1999; Virginia and Wall, 1999; Courtright et al., 2001). In another study by Porazinska and Wall (2002) it was reported that moisture showed positive correlation with *Plectus* and *Eudorylaimus* whereas a negative correlation with *Scottnema*.

PATHOGENICITY OF ENTOMOPATHOGENIC NEMATODES

Entomopathogenic Nematodes are natural enemies of insect hosts and cause disease in them. It has been reported by many researchers that EPNs kill different
instars of different hosts (Table 3.1) at different time intervals. This view is supported by the work of Divya et al. (2010) who reported that 2nd and 3rd instar of S. litura, H. armigera and Galleria mellonella died rapidly than 4th instar when treated for 24 h with 300 IJs/L of H. indica. In another study, Sankar (2009) observed that H. indica took less time to kill second and third instar of rice leafroller, C. medinalis (Guenee, 1854) as compared to final instar larvae and pupae when they were exposed for 24 h at 100 IJs/L. This shows that larval age effects differential toxicity of nematodes. With advanced age of the larvae, longer exposure time periods and high concentrations were required to produce 100% mortality. Many studies believe that vulnerability variation among different instars of host are due to variation in size and accessibility of body’s natural openings (spiracles, mouth and anus) which act as an entry gates for nematodes/juveniles (Dowds and Peters, 2002). Youssef (2014) reported that S. carpocapsae could cause 100% mortality against the desert locust, S. gregaria within 120 h of inoculation on 3rd and 4th instars. The Dauer juvenile of Steinernema carpocapsae used against Red palm weevil (P. dactylifera) proved to be effective and showed 80% efficacy (Dembilio et al. 2010). Similarly, Heterorhabditis bacteriophora showed greater pathogenecity against S. sacchari (a mealybug on the sugarcane) (Roby 2018). Chive Gnat (Bradysia odoriphaga) a rigorous pest of the Chinese Chive (Allium tuberosum) was controlled by 10 species of Steinernema and 3 species of Heterorhabditids. S. feltiae and S. hebuinse led to significant decrease in B. odoriphaga species. Heterorhabditis acted against the D1 larvae and resulted in high mortality (Jaun et. al., 2013). In another study, H. bacteriophora was lesser effective against 2nd instar larvae than S. feltiae with 45-53% mortality of grubs of B. coriacea. But showed 100% mortality against 2nd instar larvae (Sharma, 2015). Studies also claim that Plum curculio (Conotrachelus nenuphar) was controlled by S. riobreve having greater than 90% control of the weevil in 2 out of 3 trials (Shapiro-Ilan et al., 2004). In another study, it was found that the dominant Mealybug (Plonococcus ficus) pest of Grapes was controlled by H. noenieputensis causing 90% mortality and S. yirgalemense with 63% mortality. At 100% humidity, 70% mortality was also recorded (Platt et al., 2016). Heterorhabditis noenieputensis is highly susceptible for medfly larvae also (James et al., 2018). Although EPNs are used to control the pest population over the years, but recent studies also claim that mosquitoes Culex pipiens L. (Diptera: Culicidae) were also controlled by EPN species S. feltiae, S. carpocapsae and H. bacteriophora under laboratory conditions and dead mosquitoes were found after an interval of 24 h, 48 h, 72 h, 96 h and 120 h of trial (Toksoz and Saruhan, 2018). Another study states that Aldle leaf beetle which is the most destructive pest of the Oak trees was controlled by using H. bacteriophora, S. websteri and S. feltiae. The pre pupae stage of the leaf beetles was most susceptible to the EPNs than the adult stage. S. websteri controlled the population in both the stages (Bayramoglu et al., 2018). Similarly, S. carpocapsae controlled the sweet potato weevil population (Jansson et al., 1993) and Saccharococcus sacchari was controlled by the Heterorhabditis species (Roby, 2018).

In 2014, Sharifi et al. studied two commercially available entomopathogenic nematode species i.e. S. carpocapsae and H. bacteriophora and found that these nematodes showed virulence against the last instar of O. coerulescens. Le Vieux and Malan (2013) conducted study in South Africa and reported that two indigenous species S. yirgalemense and H. zealandica could cause 65 and 96% mortality respectively against
adult female of *P. ficus*. Goudarzi et al. (2015) studied the potential of two indigenous EPN species, *S. carpocapsae* & *H. bacteriophora* on *A. segetum* larval, pre-pupal and pupal stages with different nematode concentrations of 25, 50, 75 and 100 IJs/L and at different time intervals i.e. 12, 24 and 48 h. In laboratory and glass house conditions, final instar larva was most susceptible whereas susceptibility was less in case of pre pupal and pupal stages. Chongchitmate et al. (2005) also studied the biomonics of some EPN species on insect larvae of Lepidoptera. Three species of EPNs like, *S. feltiae*, *H. bacteriophora* and *S. carpocapsae* were studied for their efficacy against *T. absoluta*. It was found that they caused 76.3, 88.6 and 92% larval mortality respectively at concentration of 60 IJs/L. Under green house conditions, the foliar application of *S. carpocapsae* caused 87% whereas *H. bacteriophora* and *S. feltiae* could cause 95% mortality. Their study demonstrated that EPNs can infect larvae, pupae as well as the adults of *T. absoluta* but larval stage was the most susceptible (Batalla-Carrera et al., 2010). Similar to this work, studies on biocontrol of *S. litura* have shown that *H. bacteriophora* HY caused 100% mortality against 2nd instar after 20 h. *H. bacteriophora* HY, *S. monticola* CR and *S. carpocapsae* PC caused 100% mortality after an interval of 47 h against 3rd and 4th instar larvae. *S. carpocapsae* PC caused 100% mortality after an interval of 47 h against 3rd and 4th instar larvae of *S. litura*. Trdan et al. (2006) studied the efficacy of four nematode species (*H. megidis, H. bacteriophora, S. carpocapsae* and *S. feltiae*) on two serious pests of stored grains, *S. granaries* and *O. surinamensis*. Nematodes caused mortality of insect pests at different temperatures and concentrations (at 20°C, LC_{50} 803-1195 IJs/adult and at 25°C, LC_{50} 505-1175 IJs/adult). Radova (2010) reported that *S. carpocapsae* reacted negatively at 50 and 500 IJs/L concentration and showed decrease in efficacy to pre-colonisation. Generally, *S. arenarium* showed low efficacy against **T. molitor** larvae but after pre-colonisation, slight increase was observed. *S. feltiae* could show increase in efficacy against these larvae at 500 IJs/L. Lalramliana and Yadav (2009) evaluated the pathogenicity of *S. thermophilum, S. glaseri* and *H. indica* against the larval instars of cabbage butterfly, *P. brassicae*. They found *S. thermophilum* as the most potent nematode species which caused 100% mortality in 24 h at 50 IJs/L. Besides, *H. indica* showed 100% mortality at 100 IJs/L in 48 h while *S. glaseri* showed no mortality in 24h. Pervez (2014) studied the efficacy of *Heterorhabditis* sp. (IISR 01), *Steineriema* sp. (IISR 02 and 03), *Oscheius* sp. (IISR 04, 05, 07 and 08) and *S. carpocapsae* against hairy caterpillar, *Euproctis sp.* larvae and shoot borer, *C. punctiferalis*, larvae and pupae. All the species caused 100% mortality of *Euproctis* sp. larvae except *Oscheius* sp. But it was more lethal for *C. punctiferalis*, causing 100% mortality. Studies conducted on the efficacies of 3 native strains of EPNs such as *Heterorhabditis bacteriophora* (ZET35), *Steineriema feltiae* (ZET31), *Steineriema websteri* (AS-1) against the prepupal and adult stage of the *A. alni* have observed that pre pupal stage was more sensitive than adults (Bayramoglu et al., 2018). Adiroubane et al. (2010) evaluated the efficacy of *S. siamkayai* against *C. medinalis, E. vitella, L. orbonalis, P. xylostella* and *S. litura* and reported that susceptibility of insect larvae increased with increase in concentration. LD_{50} value decreased with increase in exposure time and LT_{50} values also decreased with increase in dose.
EPNs display good adaptability with environment and are used along with other chemicals. Studies have shown that a combination of EPNs and imidacloprid against white grub (especially scarab species) shows low environmental impact but high compatibility for biological control of turfgrass insects. Similarly, synergist combinations of EPNs and neonicotinoid insecticide were used for the remedial treatment of the white grub (especially *P. japonica* and *E. orientalis*) infestations (Koppenhofer and Fuzy, 2008). In another study, *F. occidentalis* was controlled by different concentrations of nematodes using *Crysanthemum* as model crop. A combination of EPNs and Agral 90 was sprayed on *F. occidentalis* where it was found that *S. feltiae* was most effective against Pre pupae and pupae stage (Buitenhuis and Shipp, 2005). Studies have found that Cabbage infesting moth (Diamondback moth) was controlled by *S. thermophilum* when used alternatively with insecticide (η-cyhalothrin) causing 46% mortality whereas 40.5% mortality rate with the insecticide treatment. The mortality showed increase with increase in temperature (Somvanshi et al., 2006). Different salts like NaCl, KCl and CaCl\(_2\) have different affect on entomopathogenic nematodes such as *S. glaseri* and *H. bacteriophora* differently. But in *S. glaseri* its efficiency, virulence and penetration remain unaffected by these salts. Effects of NaCl on *H. bacteriophora* impart the toxicity and possibly interfere with the host finding behavior (Thurston et al., 1994). Temperature plays a significant role in increasing the infectivity, development, reproduction and storage stability of different species of EPNs. Infectivity, development, storage stability and reproduction of *S. scapterisci* is greater at high temperature which shows that they adapt in warm climates (Grewal et al., 1993). In South Africa, under semi-field trials *Heterorhabditis zealandica* was used to study the infection of codling moth *Cydia pomonella* (L) in late instar diapausing stage, under different temperature conditions. The effect of Infective Juveniles (IJs) on codling moth larva under morning conditions was more than the evening applications, while there is no effect on larva in direct sunlight (Waal et al., 2018). Several experiments were conducted to show the effect of herbicides on infected juveniles at different time and temperature which revealed that herbicides had negative effect on EPN survival, while the use of different control ingredients (insecticides and pesticides) at the same time could reduce the cost in time consumption of pest cum weed control (Lazik and Trdan, 2017). Studies have also shown that *Heterorhabditis bacteriophora* is less susceptible on *Popillia japonica* 3\(^{rd}\) instar as the season changes from rainfall to spring (Paoli et al., 2017).

Under high humidity and moderate temperature, *Steinernema virgalemense* is highly susceptible to female *Planococcus ficus*. The mortality rate of *Planococcus ficus* was recorded as 72% at 25°C due to the application of *Steinernema virgalemense*, at 30°C, it was 45%, while at 15°C the mortality rate was 9% and at 100% relative humidity, the mortality rate is 70%. Mortality rate decreases with decreasing relative humidity levels (Platt et al., 2018). *Heterorhabditis noeniputensis* was having highest mortality rate of 90% where *Steinernema yirgalemense* was having mortality rate of 63%. *S. yirgalemense* at 100% level of relative humidity resulted in 70% mortality, whereas decreased levels of relative humidity resulted in decreases mortality (Platt et al., 2018).

Immune system of the host is also the deciding factor for nematode efficacy against insect pest. Certain reports claim that at egg stage there is low activity of detoxifying
enzymes which progressively increase with larval age and finally declines to zero at pupation stage (Ahmed, 1986; Mullin, 1988). Eicosanoid represents the oxygenated metabolites of carbon dioxide PUFA and immune responses in mammals play a key role in mediating various physiological processes. EPNs bacteria suppress the eicosanoid biosynthesis which inhibits host-insect immunity and enhance their pathogenicity (Kim et al., 2018). Studies on Heterorhabditidae nematodes have revealed that these nematodes and their bacterial symbionts prevent the phagocytic ability of haemocytes which interferes with cellular immune defence of the insect and proves fatal (Sambeek and Wiesner, 1999). Wang and Gaugler (1999) reported that S. glaseri uses SCP3a (a surface coat protein) to avoid encapsulation in Japanese beetle, P. japonica larva. SCP3a protein destroys host haemoocytes and protects elimination and detection of distinct nematode species, thus playing a significant role in establishment and survival of nematode. Granulocytes are one of the major effector cells which play an important role in recognizing self and non-self (Gotz and Boman, 1985). In Drosophila, plasmatocytes mediate phagocytosis and helps in removal of dead cells during metamorphosis, embryogenesis and pathogenesis. In response to infection, antimicrobial peptides are secreted by plasmatocytes (Agaisse et al., 2003). During infection, their number increases rapidly and is responsible for numerous cellular defences including cell spreading, cell aggregate formation, phagocytosis, nodulation and encapsulation (Strand, 2008). In another study, Ebrahimi et al. (2014) showed that PO activity increased significantly in response to S. carpocapsae infection in Colorado potato beetle, L. decemlineata. They found that pathogenic expression in case of direct injection of nematode was three times more than infection through the soil. In soil application, the reasons for delay in pathogenic response includes the time taken by the nematode to navigate the barriers like distance from the soil to haemolymph, penetration due to insect behavior and structural defences (Koppenhofer et al., 2000; Toubarro et al., 2009).

TOOLS USED FOR MOLECULAR CHARACTERIZATION OF EPNS

Many researchers have used molecular characterization as a tool for identification and phylogenetic studies of nematodes like those studied in the north-west of Iran (Kary et al., 2009), in Portugal (Valadas et al., 2014), Herault and Gard, Southern France (Emelianoff et al., 2008), Ordu Province, in Turkey (Erturk et al., 2014), Thailand (Noosidum, 2010), Kodaikanal Hills of South India (Razia and Sivaramakrishnan, 2014), Uttar Pradesh in India (Kaushik and Chaubey, 2016) and many more. Several isolates of Heterorhabditis have been identified using isozyme patterns (Akhurst, 1987), Neoaplectana glaseri and N. anomalai has been differentiated based on proteins, malate dehydrogenase, and acid phosphatase successfully. ITS-RFLP (Internal Transcribed Spacer-Restriction Fragment Length Polymorphism) region located between 18S and 26S rDNA genes are the most suitable marker for identification of EPNs including Heterorhabditids or Steinernematids (Pamjav et al., 1999).

The ITS (Internal Transcribed Spacer) region which is located between the repeating array of the nuclear 18S and 28S ribosomal genes, is a handy genetic marker among eukaryotes, including organisms as diverse as protozoa, plants, vertebrates, nematodes, and fungi. ITS data have been used in constructing phylogenetic trees, estimating genetic population structures, evaluating population-level evolutionary
processes, and determining taxonomic identity. The rDNA cistron structure largely contributes to its wider applicability. Domains of rDNA cistron evolve regularly at different rates; thus, this region can be used to address diagnostic and evolutionary problems at different levels of divergence. The rDNA is a component of middle repetitive family of nuclear DNA genome and possess multiple copies of these genes within the genome which facilitates amplification of PCR product from single juvenile as well as adult nematodes.

The use of ITS region for identification of nematodes has received a wide attention by the nematologists (Joyce et al., 1994; Vrain and McNamara, 1994; Campbell et al., 1995; Cherry et al., 1997). Most of these studies have been emphasize upon plant-parasitic species as well as on animal or beneficial insect parasites. Yet, there is no nematode species that has ever failed to give an amplification product of ITS region with “Universal” PCR primer sets. Universal amplification along with ITS amplification from various nematodes suggest that molecular method based on the rDNA ITS region can be used to identify any species, population, or ecological community of nematodes (Vrain and McNamara, 1994). 53 EPNs isolates have been discriminated successfully into Steinernema and Heterorhabditis using ITS region (Iqbal et al., 2016). A survey of EPNs in Philippines, relied upon ITS1-ITS2 of rDNA and revealed the occurrence of four EPNs successfully i.e. Heterorhabditis indica, Steinernema tami, Steinernema minutum, and Steinernema abbasi (Caoili et al., 2018).

**PHYLOGENY RECONSTRUCTION OF EPNs**

In order to trace the evolutionary history of an organism and draw a meaningful comparison (adaptation, speciation, gene flow, ecology) with other organisms always requires a robust phylogenetic tree. SSU-rDNA based phylogeny of 53 taxa suggested that EPNs belongs to clade 5 with respect to another nematode group (Blaxter et. al., 1998). Phylogenetic relationship among EPNs has been elucidated by using various datasets including morphological trait, RFLPs and RAPD (Liu et al., 1996, Reid et al., 1997). Phylogeny reconstruction of EPNs based on morphological character is tedious due to quantitative measurement of characters and its low specificity. Availability of the new molecular marker facilitates to reconstruct new and reliable phylogeny of EPNs. rRNA genes are suitable for the distinction between family and genus level but found less suitable for closely related genera of Heterorhabditis (Liu et al., 1997). Large subunit and ITS of rDNA, mitochondrial ND4, Cox1 have been widely used to develop a phylogenetic tree of entomopathogenic nematodes (Adams et al., 1998; Liu et al., 1999; Szalanski et al., 2000; Stock et al., 2001; Nguyen et al., 2001). Among all these markers, ITS, ND4 and Cox1 considered as most suitable for phylogeny tree reconstruction due to the regular and rapid rate of evolution.

**UNDERSTANDING MUTUALISTIC INTERACTION WITH BACTERIA**

EPNs associate mutually with their symbionts like Xenorhabdus and Photorhabdus to kill the targeted host. Nematode provides space in the body for bacteria to protect from the adverse environmental condition and to carry them to the host insect. In return, bacterium favors nematode in killing its host and providing a suitable medium to reproduce and develop nematode within cadaver. Understanding the basis of interaction of nematode, bacteria, and host at molecular level could be
Entomopathogenic Nematodes

helpful to understand pathogenicity perspective against other insect fauna. Every EPN species possess a specific bacterial symbiont species but nature behind the specificity is still not defined.

EPN GENOMIC RESOURCES TO INVESTIGATE PARASITISM AND MUTUALISM

The sequencing and annotation of a species genome and its transcriptomics information is often the first step in exploiting these data for comprehensive biological understanding. Heterorhabditis has been chosen over other EPNs because of its close relatedness to C. elegans, and H. bacteriophora strain TTO1 was chosen because its genome sequence was mutually associated with P. luminescens belonging to subsp. laumondii (Duchaud et al., 2003). The nematode has 77Mbp genome size and 21,250 putative protein-coding genes, and half of the protein-coding genes were new with unidentified function with no orthologues found in other related species. Similarly, 317 out of 603 predicted secreted proteins are new with uncharacterized function in addition to 9 peptidase inhibitors, 7C-type lectins and 19 putative peptidases that may function in the interactions with hosts insect or bacterial symbionts (Bai et al., 2013). While a recent article (McLean et al., 2018) have predicted 15,747 protein-coding genes by a BRAKER1/softmasked tool. The H. bacteriophora genome sequence along with sequences from other strains (e.g., GPS11) of H. bacteriophora allow Single Nucleotide Polymorphisms (SNPs) to be identified which can be used in mapping experiment for detail functional genomics aspect of a particular gene. The transcriptomic study is important as for a function of a gene is concerned. Transcriptome analysis of the H. bacteriophora strain TTO1 revealed total of 31,485 high-quality ESTs. Out of these, 12 ESTs corresponding to 8 genes potentially are involved in RNAi pathway, 22 ESTs corresponding to 14 genes potentially are involved in dauer-related processes, and 51 ESTs corresponding to 27 genes potentially are involved in defense and stress-related responses. A comparison of ESTs and proteins from free-living nematodes revealed the identification of 554 ESTs specific to parasitic nematode in H. bacteriophora including those encoding F-box-like/WD-repeat protein theromacin, PAZ domain containing protein and Bax inhibitor-1-like protein. This EST collection provides opportunities for research on this nematode-bacterium symbiotic association complex. The comparison of ESTs of H. bacteriophora TTO1 with ESTs of FLNs, PPNs and AHPNs led to the identification of 554 parasitic nematode-specific ESTs which would be helpful for future research linked to insect parasitism by the nematodes. This study also reveals that a small number of ESTs implicated in the RNAi pathway, among which is an EST encoding a Drosha homolog, suggest structurally different RNAi pathway components from those in C. elegans. Overall, new, parasitic nematode-specific and C. Elegans homologous genes were identified in this EST study, greatly facilitating gene functional analysis, genome annotation, microarray development and population genetic studies (Bai et al., 2009). The high-quality draft sequence and ESTs has rapidly broadened our knowledge about gene content, organization, structure and expression. This has enabled many studies, such as functional genomics to elucidate gene function in H. bacteriophora, as related to parasitic or symbiotic biology.
Table 3.1. List of different EPNs and their hosts reported by some authors in various studies.

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<th>EPN specie</th>
<th>Host</th>
<th>Author</th>
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<tr>
<td>S. carpocapsae</td>
<td>R. ferrugineus (date palm weevil)</td>
<td>(Dembilio et al., 2010)</td>
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<td>S. glasseri</td>
<td>S. sacchari</td>
<td>(Roby, 2018)</td>
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<td>S. krasei</td>
<td>Oscheius tipulæ and O. oniri</td>
<td>(Blanco-Perez. et al., 2017)</td>
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<td>S. feltiae</td>
<td>B. coriaceea (white grub)</td>
<td>(Isha Sharma, 2015)</td>
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<td>Steinernema glaseri</td>
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<td>S. carpoca psae</td>
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<td>Steinernema feltiae</td>
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<td>H. floridensis</td>
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<td>Steinernema riobrave,</td>
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<td>Naupactus godmani</td>
<td>(Glucu et al., 2019)</td>
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<td>H. bacteriophora</td>
<td>Fuller rose beetle</td>
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<td>H. indica</td>
<td>Diaprepes abbreviatus</td>
<td>(Borai et al., 2018)</td>
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<tr>
<td>S. feltiae</td>
<td>Dendrolimus pini</td>
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**Entomopathogenic Nematodes**

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**CONCLUSION**

Emergence of entomopathogenic nematodes as bio-control agents has revolutionized the approach adopted in integrated pest management strategies. These organisms have been isolated from different soils across the globe displaying exceptional adoptive capabilities in diverse habitats. This property enhances their acceptability among scientific community as well as farmers. Another aspect related to EPNs is their ecofriendly existence in the ecosystem. They are safe to non target organisms, plants, vertebrates and also possess a wide range of hosts (Poinar, 1989; Akhurst, 1990). They cause no serious threat to environment as well as human health which was well established by exempting them from registration in United States of America (Gorsuch, 1982). Another attractive property about EPNs is their compatibility with chemicals pesticides which has yielded more efficient results (Hara and Kaya 1983; Rovesti et al., 1988; Forschler et al., 1990; Rovesti and Deseo, 1990; Zimmerman and Cranshaw, 1990). Moreover, possession of symbiotic bacteria in their gut is another property of EPNs which links insect pathology with insect nematology (Gaugler and Kaya, 1990). Results from experiments in laboratory conditions are convincing but field results are not up to expectations. Molecular insights into EPN genome and the popularity of biomarkers have further raised their importance in context of human welfare. Need of the hour is to enhance industrial production of more and more isolates and extensive research in the field conditions. Over the years studies have explored many new research dimensions with prime focus on recent advances to critical issues. In near future EPNs will lead the approach to control insect pest population through biological control strategies.
FUTURE PROSPECTS

EPNs have yielded good results in laboratory bioassays and are popularly used in many countries against different pests (Table 3.1). But the field results are not at par with the *ex situ* studies. Moreover intensive search for indigenous species of EPNs should be encouraged on a large scale. Further studies are required to understand nematode invasion and host susceptibility along with new dimensions to exploit EPNs to their full potential.

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