PATHOGENICITY OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES AGAINST SELECT INSECT PESTS

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(2017-11-092)

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PATHOGENICITY OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES AGAINST SELECT INSECT PESTS

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2019

DECLARATION

I, hereby declare that this thesis entitled **"PATHOGENICITY OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES AGAINST SELECT INSECT PESTS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
@	At the rate of
CD	Critical Difference
EC	Emulsifiable concentrate
EPN	Entomopathogenic Nematodes
et al	And others
g	Gram
ha^{-1}	Per hectare
HAT	Hours After Treatment
IJ	Infective Juvenile
KAU	Kerala Agricultural University
kg	Kilogram
l	Litre
mg	Milligram
ml	Millilitre
µl	Microlitre
%	Percentage
SC	Suspension Concentrate
sp.	Species
SD	Standard Deviation
viz.,	Namely
i.e.,	That is

rpm	Rotations per minute
h	Hour
min	Minutes
s	Seconds
V	Volts
mA	Milli Ampere
°C	Degree Celsius

Introduction

1. INTRODUCTION

Food security in mankind is facing a serious threat due to several biotic and abiotic factors. Crop yield losses due to biotic factors include insect-pests, diseases, weeds, nematodes and rodents that range from 15-25 per cent in India, among which 60-70 per cent loss is due to insect pests which are of great concern. Pest management in agriculture is a challenging task in the context of increasing productivity without deteriorating the environment. But the consumption of pesticides has been showing an increasing trend in the country and accounts for 60282 metric tons (Rajendraprasad *et al.*, 2016). In Kerala, the pesticide consumption is reported as 856 metric tons which accounts for per ha consumption of 0.41 kg/ha (Devi and Nath, 2016). Even though insecticides are very useful against insect pests, they are posing problems like environmental pollution, pesticide resistance, pest resurgence, residue in feeds, food, soil and water and some socio economic problems. So it is utmost necessary to have a sustainable use of chemical pesticides in controlling these insect pests for maximizing the crop yield and meeting the increased demands of agricultural products and commercialization. Thus the demand for biocontrol agents has been increased to overcome these problems.

Biological control of crop pests has been successfully applied in Integrated Pest Management and also as a component in organic farming due to their target specificity, eco safety, non-development of resistance, reduced number of applications, yield and quality improvement. Naturally occurring entomopathogens are important biotic factors for suppressing the populations of insects. One among the biocontrol agents are the Entomopathogenic nematodes (EPNs) which is gaining momentum.

EPNs are a group of nematodes causing death to insects. They belong to families Steinernematidae, Heterorhabditidae and Rhabditidae. The genus under these families include *Steinernema/Neosteinernema*, *Heterorhabditis* and *Rhabditis* (*Oscheius*) and

have a symbiotic association with insect pathogenic bacteria belonging to the genera *Photorhabdus, Xenorhabdus* and *Serratia* respectively. These nematodes have a life cycle consisting of egg stage, four juvenile stage and adult stage. The 3rd stage juvenile is known as infective juvenile (IJ) which is the only free living stage found outside the host body and is found in soil and is activated by insect movement and then follows a gradient of CO_2 to find the insect larvae by 'Cruise' and 'Ambush' foraging to get entry into the insect's body cavity in order to kill it. EPNs enter through the insect's natural body openings, the mouth, anus or respiratory openings (spiracles) and then penetrate into the haemocoel from the gut. Once on reaching the insect's blood, IJ releases the highly specialized symbiotic bacterium. The bacteria then convert the insect into suitable food for the nematodes and produce a range of antibiotics (Akhurst and Bedding, 1986) that preserve the dead insect from putrefaction while the nematodes feed and reproduce in it. The death of the insect occurs usually within 24-72 hours. Depending upon the host size, the nematode completes 1-3 generations. Upon the depletion of food sources in the host cadaver, a new generation of IJ is produced and comes out from the host cadaver into soil for searching new hosts.

EPNs are preferred over other biocontrol agents as they have ability to search the target insect leading to quick kill than any other microbial control agents and have a broad host range infecting Coleoptera (F: Cerambycidae, Chrysomelidae, Curculionidae, Scarabidae), Diptera (F: Tephritidae and Agromyzidae), Lepidoptera (F: Noctuidae, Pyralidae and Sesiidae) and Orthoptera (F: Gryllotalpidae and Acrididae) (www.nbair.res.in).They are even compatible with many pesticides, give a long-term control and are exempted from registration considering it as one of the best microbial control measure for the management of insect pests.

As the information pertaining to different indigenous species of entomopathogenic nematodes effective against different pests prevalent in Kerala is meager, the present study envisages to:

1. Identify indigenous entomopathogenic nematodes prevalent in Kerala
2. Evaluate their pathogenicity to termites, lepidopteran and coleopteran pests

Review of Literature

2. REVIEW OF LITERATURE

The yield of vegetables in the tropical and subtropical regions is substantially reduced due to the heavy infestation of insect pests. They reduce the photosynthetic efficiency of the plant by feeding on the leaves and other foliar parts and decrease the nutrient uptake by damaging the roots leading to a massive reduction in the production of vegetables. As a result of this, growing concern among farming community has led to indiscriminate use of pesticides. Due to its toxic effect, not only the pests but also other non-target organisms are affected. The demand for biocontrol agents has been thus increased to overcome the problems of pesticides. One among the biocontrol agent is the Entomopathogenic Nematodes (EPN) which has been found effective in controlling insect pests and is gaining momentum. EPNs can be mass produced easily and applied using conventional spray equipment. The present study envisages to identify indigenous EPNs prevalent in Kerala and to evaluate their role as bio control agents in the management of insect pests.

Literature pertaining to isolation and identification of entomopathogenic nematodes and its pathogenicity against insect pests are presented here.

2.1 ISOLATION AND MORPHOLOGICAL IDENTIFICATION OF EPNS FROM SOIL

EPNs have been well known since 1923, when Steiner identified the species *Aplectana kraussei*. Steiner later described a new species, *Neoaplectana* (=*Steinernema*) *glaseri* Steiner, from Belgium as a natural pathogen of *Hoplia philanthus* Füessly (Steiner, 1929). Glaser and Fox (1930) identified this nematode infecting grubs of the Japanese beetle, *Popillia japonica* Newman at the Tavistock Golf Course near Haddonfield, New Jersey, USA. A new species of EPN, *Heterorhabditis bacteriophora* Poinar, was described in 1975, from a new genus and family Heterorhabditidae of Rhabditida (Poinar, 1975). In the last few decades, many new EPN isolates and species have been discovered from different habitats all over

the world (Hominick, 2002). A new species of EPN, *Oscheius* have been found to parasitize insect hosts using pathogenic bacteria (Nguyen and Hunt, 2007). Currently, 118 and 20 species of *Steinernema* and *Heterorhabditis* respectively have been described from different habitats around the world (Hunt and Sergei, 2016).

Southey (1970) collected soil samples to recover EPN directly from the soil through sugar floatation, Baermann funnel and mist extraction techniques. A new technique was adopted by Bedding and Akhurst (1975) using *Galleria mellonella* Linnaeus larvae as trap insect for collecting EPNs indirectly from the infected hosts. Beavers *et al.* (1983) collected EPNs from naturally infected host insects.

In Japan, *Steinernema kushidai* Mamiya, was first isolated in Shizuoka from a tree nursery where an outbreak and an infestation of white grubs had occurred (Koizumi *et al.*, 1987). Nguyen and Smart (1990) isolated *S. scapterisci* Nguyen and Smart, for the first time from the mole cricket, *Scapteriscus vicinus* Scudder in Uruguay. Gardner *et al.* (1994) conducted a survey for studying the soil EPNs of the Hawaiian islands and described *H. hawaiiensis* (now regarded as the junior synonym of *H. indica*). Stock *et al.* (1996) conducted a survey of soil-dwelling insect pathogens in northern and southern California and recovered *H. hepialus* (now considered as *H. marelatus* after Stock) from ghost moth caterpillars (*Hepialis californicus* Boisduval). Phan *et al.* (2003) conducted a survey of EPNs in Vietnam and three isolates were collected from forests of Backan, Ninhbinh and Kontum provinces and described a new species *H. baujardi*.

Hazir *et al.* (2003) conducted an extensive soil survey in Turkey and recovered 22 positive samples out of 1080 and 15 were identified as *Steinernema* isolates and 7 were *Heterorhabditis* isolates (*H. bacteriophora*, *S. feltiae* Filipjev, *S. affine* Bovien). Tabassum *et al.* (2005) conducted a survey in Pakistan from cultivated land of fruits and vegetable and found that out of 603 samples collected, 16 samples showed positive results. From these 12 isolates belonged to the genus

Steinernema and 4 isolates belonged to the genus *Heterorhabditis.* A survey was conducted for EPN in oak-juniper woodlands of south eastern Arizona and found that out of 120 soil samples, 23.3% were positive for EPNs where 78.5% was *Steinernema* spp. and 21.5% was *Heterorhabditis* spp. (Stock and Gress, 2006).

Kary *et al.* (2009) collected soil samples from different locations in Iran and baited with *G. mellonella* larvae found that out of 833 soil samples, 27 were positive, with 17 containing *Heterorhabditis* and 10 having *Steinernema* isolates. Khatri-Chhetri *et al.* (2010) observed the natural occurrence and distribution of EPN in Nepal and recovered 29 EPN isolates out of the 276 soil samples. Among 29 positive samples, 7 samples yielded *H. indica* Poinar, and 22 samples contained *Steinernema* sp. with a distribution frequency of 24.14 and 75.86 per cent respectively.

Out of 105 soil samples collected from rhizosphere of lemon, cotton and mung bean of IARI, New Delhi, 15% soil samples were positive for *Steinernema* sp. and *Heterorhabditis* sp. (Ganguly and Singh, 2001). Hussaini *et al.* (2001) conducted a survey in India and isolated 3 *Steinernema* spp. using *Galleria* baiting technique which were later identified as *S. tami* and *S. abbasi.* As a part of studying bio-diversity of EPN in Rajasthan, out of 707 samples collected, 14 samples found positive to EPN of which 6 samples yielded *Heterorhabditis* spp. and 8 samples yielded *Steinernema* spp. (Parihar, 2002).

Prasad *et al.* (2001) collected 139 soil samples from 10 localities representing cultivated area, forest, scrub land and coastal sandy region of South Andaman and reported a recovery rate of EPNs as 11.51 per cent. A total of 16 *Heterorhabditis* isolates were recovered (14 from coastal sites and 2 from inland sites).

Mohandas *et al.* (2004) isolated a pathogenic *Rhabditis (Oscheius)* sp. from the grubs and pupae of sweet potato weevil and was found to be effective against arecanut spindle bug, *Carvalhoia arecae* Miller and China and rice yellow stem borer, *Scirpophaga incertulas* Walker.

As a part of survey of EPNs in Kerala, 430 soil samples were collected from coconut plantations, undisturbed areas, forest land and cowdung pits of 7 district of Kerala. Out of these samples, 129 (30%) were positive for EPN. *H. indica* occurred in 128 samples (99%) and *Steinernema* sp. in only one sample (0.8%) (Sosamma and Rasmi, 2002). In Kerala, Abbas (2010) isolated *H. indica* and *S. abbasi* from red palm weevil, *Rhynchophorus ferrugineus* Olivier, infesting date palms.

Ali *et al.* (2005) collected 496 soil samples from rhizosphere of pod borer endemic territories of pigeon pea and found 8 (1.6%) positive for the presence of EPNs. The most effective isolates were identified as *S. masoodi* and *S. seemae* isolated from Kanpur and Hamirpur areas respectively. Another strain of *S. masoodi* was isolated from Aligarh and was assigned as *S. masoodi* AMU EPN-1 (Khan and Uzma, 2007), but now all these species are considered as species inquirenda due to poor description of species.

H. indica and *S. glaseri* application at 100 IJs per insect resulted in 80 per cent mortality in *Cosmopolites sordidus* Germar and *Odoiporous longicollis* Oliver under *in vitro* condition. Mortality of weevils was 52 to 56 per cent inside the banana rhizome (Remya, 2007).

Out of 250 soil samples collected from different locations of Guntur district, three samples (1.2%) were found positive to EPNs and they were morphometrically identified as *S. karii* Waturu and *H. indica* (Subbanna *et al.*, 2008).

Khan and ZialHaque (2010) conducted a survey in four districts of Western U.P. Out of 231 soil samples 38 samples were positive for EPNs of which 32 samples (84.20%) contained Steinernematids and 6 samples (15.78%) contained Heterorhabditids. The frequency was recorded highest in Aligarh (31.6%), followed by 26.2% in Bulandshahr and 21.1% in Moradabad.

Vasanthi *et al.* (2014) gathered 110 soil samples from the cashew plantations of the Directorate of Cashew Research and isolated six effective native strains of EPN using insect bait technique against cashew stem borer, *Plocaederus* sp. These EPNs were identified by using molecular tools and found that four isolates matched with *H. bacteriophora* and two with *S. abbasi.*

Pervez *et al.* (2014) gathered 202 soil samples from ginger ecosystem of different districts of Kerala and found seven samples positive towards EPN. Morphometric and morphological characterization revealed that out of the 7 native strains of EPNs, three species belong to genus *Steinernema*, one *Heterorhabditis* and three *Oscheius.*

Eleven EPN isolates were obtained using Galleria baiting technique from 436 soil samples collected from different locations of Meghalaya and were identified as *H. indica*, *Steinernema* sp. and *Mermithid* sp. (Devi, 2008). *Heterorhabditis* was observed as the predominant isolate in 131 EPN isolates obtained from 930 soil samples of ten districts in Himachal Pradesh (Vashisth *et al.*, 2015).

A recent survey conducted by Anes *et al.* (2018) reported that out of 141 soil samples collected from Kollam, Pathanamthitta and Alappuzha, 13.5% were found positive for the presence of EPN. They reported that soil samples collected from plots of ICAR-CPCRI, Kayamkulam recorded 33.3% of total number of EPN isolates. They isolated a new strain of *Steinernema hermaphroditum* from Pathiyoor, Alappuzha and reported it for the first time in South India.

2.2. MORPHOLOGICAL AND MORPHOMETRIC CHARACTERS OF EPN

Nguyen and Smart (1990) described *S. scapterisci* from Uruguay for the first time. The body length of IJs is 572 µm (517-609). Excretory pore was anterior to nerve ring with D% of about 38 µm and mucron was present in adults of both first and second generation males. Males had a long spicule with a prominent shaft and lamina with a small velum, tail tapering with a pointed tip. In females, excretory duct was prominent having an elliptical structure. Epiptygma was very large and well developed. It was first released in Florida in 1985 to suppress mole crickets.

Nguyen and Smart (1992) identified another species of EPNs *S. neocurtillae* from USA. In this EPN, Excretory pore was found close to head region. D% was near to 19 µm (13-26). Mucron was absent in males of second generation, spicule head was one third of spicule length. GS was about 89. Gubernaculum was three-fourth the length of spicule.

Anis *et al.* (2002) isolated *S. asiaticum* from soil samples of Pakistan and Sri Lanka. The body length of the IJs was found between 362-452 µm. The lateral fields had seven longitudinal ridges. Mucron was present in both generation females and in second generation males. The shape and size of the spicule, gubernaculum, and the arrangements of genital papillae of the first and second generation males were distinct.

Qiu *et al.* (2004) recovered a new species of EPN *S. guangdongense* from the soil sample of Jijia town in the western parts of the Guangdong province, the Peoples Republic of China. This nematode is close relative to *S. longicaudum*. Body diameter of the new species was larger and the value of EP, NE and body length/body width ratio were smaller and tail had a dorsal constriction. In males, the new species had longer spicule with short manubrium, shaft not prominent and spicule tip not tapering. The ratios 'SW' and 'GS' were also smaller. In female, the a small double

flapped epiptygma was present with a small projection on dorsal side of the tail tip and prominent post anal swelling.

Anes *et al.* (2018) isolated *S. hermaphroditum* for the first time from South India. Male, female and hermaphrodite lengths were 2368, 8365 and 922µm respectively. Males had a smaller body diameter compared to females (130 vs 289µm). Both males and females had shorter tail lengths with 40 and 68µm respectively. Males had a D% value of 47 and SW% as 133. H% of IJs was found as 51.

2.3. MOLECULAR CHARACTERIZATION

Reid and Hominick (1992) reported that the clones of a specimen have the potential to be used as a species identification tool after cloning the rDNA repeat unit obtained from a Steinernematid nematode.

Roosien *et al.* (1993) reported that only one-fifth of an IJ is sufficient to generate reproducible random amplified polymorphic DNA (RAPD) markers and thus the amplification from single juvenile doesn't require DNA isolation.

Heterorhabditis and *Steinernema* from Ireland and Britain were characterized by isoelectric focusing, DNA restriction and cross-breeding methods and did the RFLP analysis of the ITS (internal transcribed spacer) region of the ribosomal DNA repeat unit and reported that each species yielded its own unique restriction fragment length polymorphisms (RFLP). They even constructed a phylogenetic tree and concluded that RFLPs of different species identified were suitable for taxonomic purposes (Griffin *et al.*, 1994).

Anis *et al.* (2002) described *S. asiaticum* isolated from Pakistan by DNA examinations using RFLPs of the ITS region and found that rDNA repeat units of this new species is different from other species of *Steinernema*. Umarao *et al.* (2002) compared the genetic relatedness of *S. thermophilum*, a species described from India

is well adapted under high temperature conditions with four other native isolates by using RAPD markers and found *S. thermophilum* was genetically different from other native isolates. They analyzed that RAPD analysis is an excellent tool for assessing the genetic variability among different species of EPN in India.

An isolate of *S. hermaphroditum* reported from Indonesia was distinguished from other isolates using the molecular evidence obtained from ITS rDNA RFLP profiles, 28S rDNA sequence analysis, and phylogenetic reconstruction and thus helped the EPN to be established as a new species (Chaerani and Stock, 2004).

Susurluk and Toprak (2005) identified three EPNs *viz., S. carpocapsae, S. feltiae* and *H. bacteriophora* from Turkey using molecular analysis by PCR-RFLP of the ITS region.

Duncan *et al.* (2006) described a new species *S. diaprepesi* Duncan, which was found naturally infecting *Diaprepes abbreviatus* Linnaeus using de Man's formula and was distinguished from the three closely related species *S. feltiae, S. glaseri,* and *S. oregonense* by analyzing their DNA sequence at the ITS region.

S. carpocapsae was identified for the first time in Belgium by the ITS-rDNA sequence study (Ansari *et al.*, 2007). A molecular analysis of EPNs isolated from the tropical rainforest in Brazil was conducted and identified the strains LPP1, LPP2 and LPP4 as *H. indica* and LPP7 as *H. baujardi* using ITS sequence data. Further analysis of the ITS1 sequence of *H. indica* and *H. baujardi* showed a polymorphic site for the restriction enzyme Tth111 which were used to distinguish the two species (Dolinski *et al.*, 2008).

Ibrahim *et al.* (2010) did molecular and biochemical characterization of the nematode-insect relationship between four nematode species, viz. *S. glaseri, S. carpocapsae, H. bacteriophora* and *H. megdis* with the third instar larvae of white grub (*Pentodon bispinosos*) and found that some additional bands were induced due

to nematode infection and esterase activity was also found to increase after 48 hrs of infection. 65.35% and 66.2% genetic polymorphism was obtained with 104 and 80 bands for genus *Steinernema* and the *Heterorhabditis*, respectively. Kary *et al.* (2010) identified an EPN strain as *S. bicornutum* and noticed differences in ITS-rDNA PCR-RFLP with the Iranian isolate, i.e. IRA7. Later it was clarified that these molecular differences are intraspecific variations and concluded that the Iranian isolate was another isolate of *S. bicornutum*.

Spiridonov *et al.* (2011) reported a new species, *S. schliemanni* of the 'monticolum' group from Europe which differed in at least 115 positions of the ITS rDNA sequence (18%) with other species of the 'monticolum'-group. Khatri-Chhetri *et al.* (2011) placed *S. lamjungense* in the arenarium-glaseri-karii-longicaudum group on the basis of ITS-rDNA sequence analysis.

Malan *et al.* (2011) reported *S. yirgalemense* for the first time in South Africa and also reported *S. citrae* as the second new steinernematid from the same place. *H. zealandica*, *H. bacteriophora* and an unknown species of *Heterorhabditis* was also identified on the basis of sequencing and characterisation of the ITS region.

Akyazi *et al.* (2012) carried out the sequence analysis of the ITS regions of ribosomal DNA of EPN isolates to identify them as *H. bacteriophora* and *S. feltiae* and reported them for the first time from Nigeria.

Razia and Sivaramakrishnan (2014) molecularly characterized *S. siamkayai* and *H. indica* isolated from Tamil Nadu by analysis of the ITS rDNA sequences. Darissa and Iraki, 2014 characterized the ITS regions of six *Steinernema* isolates and found that different rDNA regions of these *Steinernema* isolates have different evolutionary rates at the species level but not among different isolates of the same species.

2.4 PATHOGENICITY OF EPNS AGAINST INSECT PESTS

2.4.1 Coleopteran Pests

2.4.1.1 *White Grubs (Coleoptera: Scarabidae)*

Ganguly and Singh (2001) conducted an experiment to study the efficacy of *H. bacteriophora* against *Holotrichia consanguinea* Blanchard after 4 days of exposure using filter paper impregnation method and found that the LD_{50} values for instars I-III were 110, 326 and 989 IJs/grub, respectively but the LD_{50} values differed in soil inoculation method were very high as 1875, 5097 and 8942 IJs/grub respectively. The effectiveness of four EPNs *viz., H. indica, S. glaseri, H. bacteriophora* and *S. riobrave* against grubs of *H. serrate* Fabricius was analysed in sugarcane and reported that the lowest LD_{50} value was recorded for *S. glaseri* (113.3 IJs/pupa) followed by *H. indica* (127.0 IJs/pupa) and the lowest LT_{50} value was recorded for *S. glaseri* (24.9 h) followed by *H. indica* (27.3 h) at 1000 IJs/pupa (Sankaranarayanan *et al.*, 2006). They also reported that when *H. indica* was dispensed either through soil application or by injection of IJs into the haemocoel showed 100 per cent mortality of adult beetles within 4 DAT.

Chandel *et al.* (2005) reported that *H. indica* when mixed with FYM and applied in potato, cent per cent mortality of second instar and 80.76 per cent mortality of third instar white grubs, *Brahmina corecea* Hope were observed after 28 days of inoculation under laboratory conditions. *S. carpocapsae* and *H. indica* when treated against different developmental stages of *B. coriacea* showed significant mortality by *S. carpocapsae* (68 to 93 per cent) and *H. indica* (39 to 71 per cent) after 7 day of treatment at three dosages *viz.*, 500, 1000 and 2000 IJs/100g soil in laboratory studies. Field study showed that all three doses were effective in reducing the grub population by *H. indica* (66 to 80 per cent) and *S. carpocapsae* (83 per cent) with minimal plant damage and tuber damage (Sharma *et al.*, 2009).

A study conducted on efficacy of *S. feltiae* against third instar grub of common cockchafer, *Melolontha melolontha* Linnaeus, in a laboratory experiment at 20 and 25°C in four different concentrations: 0, 250 IJs m^{-2}, 500 IJs m^{-2} and 1000 IJs m^{-2} and found 34 per cent mortality at 20°C whereas only 12 per cent mortality was achieved at 25°C. The highest mortality (53 per cent) was observed for 1000 IJs m^{-2} at 20 °C (Laznik *et al.*, 2009).

In a soil and filter paper bioassay, Bhatnagar (2011) evaluated *H. bacteriophora* against the life stages of *Maladera insanabilis* Brenske for 24 h with a high inoculation dose of 5000 IJs/90 eggs/arena of 50 mm diameter and observed that no nematode infection was developed in eggs in soil as well as on filter paper. The experiment found that adults of *M. insanabilis* were found to be more susceptible than pupae with LD_{50} values of 16796.42 IJs/100g soil/adult and 19896.60 IJs/100g soil/pupae in soil assay and LD_{50} values of 135.80 IJs/adult and 149.61 IJs/pupa in filter paper assay. The adults died earlier with a lesser LT_{50} value of 5.41 days and pupae with LT_{50} value of 5.89 days.

2.4.1.2 *Japanese Beetle, Popillio japonica Newman*

Glaser (1932) discovered the nematode which had the ability to kill an insect host which was later named as *S. glaseri* and was found to be infecting *P. japonica.*

Koppenhofer and Fuzy (2004) reported that *H. bacteriophora* treated at 100 IJs/larva caused 30 per cent mortality of the second instar larva of *P. japonica* within 7 DAT and *S. minuta* strain MP10 when observed 5 and 15 DAT caused only 16.5 and 43.9 per cent mortality respectively of the second instar larva at 100 IJs/larva whereas *S. scarabaei* showed 43 and 65 per cent mortality of the second instar larva at 7 and 14 DAT respectively at 20 IJs/larva.

Power *et al.* (2009) collected different instars of *P. japonica* from the fields of Wooster, USA and exposed them at different concentrations of 0, 10, 33, 100, 330 or 1000 IJs/grub of *H. bacteriophora* strain GPS11 and reported that higher control

was seen in October than in August or September due to the more favorable temperature for nematode activity and the presence of more susceptible larval stages and suggested that early nematode application may also provide an opportunity for nematodes to recycle and cause secondary infection.

2.4.1.3 *Banana Rhizome Weevil, Cosmopolites sordidus Germar*

Mwaitulo *et al.* (2011) tested the virulence of nine EPN isolates which were isolated from the coastal areas of Tanzania and was tested against larvae and adults of *C. sordidus* and found that adult stages were resistant to infection whereas the larval stages were susceptible. Penetration, establishment and mortality were found to increase significantly with increasing nematode dose indicating a bio control strategy against the rhizome weevil.

Bortoluzzi *et al.* (2013) conducted an experiment using native isolates of EPN for studying their interaction with insecticide (carbofuran) and found that the most virulent isolates were IBCBn24 and IBCBn40 causing mortality of 33.3 per cent and 36.7 per cent and observed the maximum multiplication and emergence from the corpse of *C. sordidus* when treated at the rate of 100 IJs cm^{-2} of psuedostem. The insecticide were not reported to affect the viability of the isolate but reduced its infectivity, although it did not affect the development of the symbiotic bacterium.

Ndiritu *et al.* (2016) conducted a study to analyse the efficacy of three Kenyan EPNs *S. weiseri*, *S. yirgalemense* and a new *Steinernema* spp. against adults and grubs of pseudostem weevil. Adults were treated with 500, 750 and 1000 IJs/adult in petri dishes and 1000, 3000 and 5000IJs/adult in pseudostems. The treatments were 300, 400 and 500 IJs for the grubs in petridishes. The adults were not susceptible while larvae were highly susceptible to the native EPNs at all concentrations causing 90 per cent larval mortality within 48 h.

2.4.1.4 *Pseudostem Weevil, Odoiporous longicollis Oliver*

Jayasree (1992) reported cent per cent mortality of pseudostem weevil grubs when infected with DD-136 (*S. carpocapsae*) at 10 days after inoculation. Padmanabhan *et al.* (2002) reported that *O. longicollis* third-instar grubs treated with 10-70 and 80-100 IJs/grub caused 33.3 and 66.6% mortality 72 HAT.

Mwaitulo *et al.* (2011) conducted a study by providing different doses of 100, 500 and 1000 IJs/grub in a cylindrical piece of pseudostem and reported that with increase in native nematode dosage the banana weevil mortality increased. But Padilla-cubas *et al.* (2010) reported that the rate of mortality did not increase with increase in dosage of IJs.

2.4.1.5 *Sweet Potato Weevil, Cylas* sp.

Ekanayake *et al.* (2001) conducted an efficacy study of two EPNs *H. megidis* and *S. feltiae* against the potato weevil, *Cylas formicarius* Fabricius, under laboratory conditions in Sri Lanka and found that *H. megidis* caused 80-90 per cent larval mortality whereas *S. feltiae* produced 70-80 per cent larval mortality at 72 HAT.

Nderitu *et al.* (2009) conducted an experiment to study the efficacy of *S. karii* and *H. indica* against *Cylas puncticolis* Boheman and reported that both EPNs significantly suppressed emergence of adult weevils from the tubers, reduced the number of pupae and also improved the tuber quantity and quality. It was also found that the EPNs persisted in the soil for more than three months after their release.

Gapasin *et al.* (2017) isolated native EPN isolates from 13 sweet potato growing areas using the insect baiting method and their distribution supports the possibility of exploiting them in an IPM management approach as biological agents against the sweet potato weevil.

2.4.1.6 *Rhinocerous Beetle, Oryctes rhinoceros Linnaeus*

Jagadeesh and Subhaharan (2014) conducted an experiment to study the effect of *S. carpocapsae* and *H. indica* against neonate and 3rd instar grubs of *O. rhinoceros* and found that mortality of third instar grubs required more number of IJs compared to neonate grub and mortality of both grubs were found to increase with increase in the days of exposure. *O. rhinoceros* was found more susceptible to *H. indica* than *Steinernema* sp. as *H. indica* was recovered from the dead cadavers after treatment of a mixture of *H. indica* and *Steinernema* sp. (Sosamma, 2003).

2.4.1.7 *Cardamom Root Grub, Basilepta fulvicorne Jacoby*

Cardamom rhizosphere was treated with *H. indica* at 100 IJs/grub during evening hours for bio suppression of root grub. In soil column bioassay, LD_{50} value of *H. indica* was found to be 49.73 IJs at 120 HAT against cardamom root grub (Josephrajkumar *et al.*, 2005).

Varadarasan *et al.* (2011) isolated local strains of EPN from cardamom niche and conducted trials in farmer's plots to test its efficacy against *B. fulvicorne* and found reduction in root grub population ranging from 71.43 to 93.38 per cent in EPN treated plot. Among the different EPN application methods, EPN infected cadaver at plant base @ 4 cadavers/plant showed a higher percentage of grub reduction ranging from 72 to 99.6 per cent.

Among the eight native EPN strains, *Oscheius gingeri* (IISR-EPN 07) and *Heterorhabditis* sp. (IISR-EPN 01) caused cent per cent mortality and had an LC_{50} value of 48 IJs/grub against *B. fulvicorne* after 72 h of exposure. (Pervez *et al.*, 2016).

2.4.1.8 *Storage Pest*

Trdan *et al.* (2006) conducted a laboratory experiment to study the bioefficacy of *S. feltiae*, *S. carpocapsae*, *H. bacteriophora* and *H. megidis* at three different doses (500, 1000 and 2000 IJs/adult) and reported that all the isolates were highly

effective against *Sitophilus granarius* Linnaeus, at 20 and 25°C with an LC_{50} value of 803-1195 IJs/adult and 505-1175 IJs/adult respectively after 7 days of exposure. *Oryzaephilus surinamensis* Linnaeus, was also found susceptible at 20°C with an LC_{50} value of 921-1335 IJs/adult.

2.4.1.9 *Others*

H. indica in a laboratory experiment caused a cumulative mortality from 66.67 to 91.67 per cent to grubs of blue beetle, *Leptispa pygmaea* Baly, at concentrations of 51 to 91 IJs/grub (Karthikeyan and Jacob, 2009).

Ali *et al.* (2012) studied the efficacy of *S. carpocapsae*, *S. feltiae*, *H. indica* and *H. bacteriophora* against mustard beetle, *Phaedon cochleariae* Fabricius, and reported that *S. carpocapsae* had the maximum multiplication of IJs per larvae at 25°C as compared to other nematodes and also showed maximum larval mortality of 90 per cent in 24 h. *H. indica* showed 97.5 per cent larval mortality when treated at 30°C after two days of exposure.

2.4.2 Lepidopteran Pests

2.4.2.1 *Diamond Back Moth, Plutella xylostella Linnaeus*

The infectivity of entomopathogenic nematodes on *P. xylostella* was studied by Baur *et al.* (1998), who reported that mortality caused by EPN was higher for early instar larvae of *P. xylostella* than late 3rd instar larvae. Suyanto and Hansen (1999) reported that the effective nematode density of *Heterorhabditis* sp. against *P. xylostella* as 450 IJs/ml of water. Shinde and Singh (2000) reported that out of eight EPN strains tested against *P. xylostella*, *H. bactertiophora* showed the maximum emergence of 271.4 IJs/mg host body weight and was the most virulent one with minimum LD_{50} and LT_{50} value of 9.16 IJs/larva and 43.26 h respectively.

Ganguly and Singh (2001) reported *S. thermophilum*, a drought resistant EPN in India. *S. thermophilum* @ 3000 and 2000 IJs caused 46 and 40 per cent mortality in *P. xylostella* respectively (Somvanshi *et al.*, 2006).

Out of five different Kenyen EPN strains evaluated against *P. xylostella viz.*, *S. karii*, *H. indica*, *S. weiseri*, *Heterorhabditis* sp and *Steinernema* sp., *S.karii* was found to have a significantly higher ET_{50} (Exposure time 50) values than that of other isolates. ET_{50} of these EPN strains ranged from 20.27-38.12 h (Nyasani *et al.*, 2007).

Gupta *et al.* (2009) evaluated field efficacy of *S. carpocapsae* and *H. indica* and found that mortality due to *S. carpocapsae* against *P. xylostella* at 2 billion IJs ha^{-1} increased from 12.5 per cent one day after application to 52.3 per cent fourteen days after treatments.

Among the five Ethiopian EPN isolates, *Steinernema* sp. HI caused 33.33, 56.67 and 82.64 per cent mortality and *Heterorhabditis* sp. AEH caused 26.67, 63.33 and 91.67 per cent mortality of *P. xylostella* larvae with 400 IJs ml^{-1} at 24, 48 and 72 HAT (Tolera *et al.*, 2016).

2.4.2.2 *Cut Worm, Agrotis sp.*

Hussaini *et al.* (2000) conducted sand column assay to study the efficacy of EPN strains against larvae and pupae of *Agrotis segetum* Denis and *Agrotis ipsilon* Hufnagel. They found that *S. bicornutum* (PDBC 3.2) and *H. indica* (PDBC 13.3) showed cent per cent mortality of larvae and pupae of *A. segetum. H. indica* (PDBC 6.71) and *S. carpocapsae* (PDBC 66.1) gave cent per cent mortality of larvae and pupae of *A. ipsilon*. The performance of all nematode populations was found to be better in sandy loam soil than in sandy soil at 5 cm depth. In another study to test the efficacy of different formulations of *S. carpocapsae*, *S. abbasi* and *H. indica* against *A. ipsilon* , alginate formulation yielded maximum mortality (60-80 per cent) in soil

assay and 33-47 per cent mortality in filter paper assay after 96 h of inoculation (Hussaini *et al.*, 2003).

S. carpocapsae @ 2,00,000 IJs/m^2 was as effective as cypermethrin at any of the concentrations in causing larval mortality of *A. segetum* (Lopez Robles and Hague, 2003). Mathasoliya *et al.* (2004) reported that *A. ipsilon* population significantly reduced on application of *S. riobrave* and plant damage in potato crop was also found to reduce from 28.29 per cent to 10.92 per cent within six days of treatment. Shapiro-Ilan *et al.* (2005) compared the pathogenicity of newly discovered *H. mexicana* (MX4) with *S. carpocapse* and observed a higher mortality per cent of *A. ipsilon* larvae with *S. carpocapsae* than *H. mexicana* (MX 4).

Fetoh *et al.* (2009) conducted a laboratory experiment to evaluate the efficacy of *S. carpocapsae* and *H. bacteriophora* against *A. ipsilon* larvae and observed that both were found more effective at 100 IJs than at 25 IJs/larva indicating that mortality increases with increase in dosage. Chandel *et al.* (2009) conducted an experiment to test the potential of *H. bacteriophora* against 3rd, 4th and 5th instar larvae of *A. segetum* by application of 1000-5000 IJs/kg of soil and reported that 1000 IJs/kg initiated infection and caused 61.3 per cent mortality of fifth instar larvae. Increase in exposure time and age of larvae increased the larval mortality.

2.4.2.3 *Tobacco Caterpillar, Spodoptera litura Fabricius*

A pathogenicity study conducted using *S. glaseri* and *H. minutus* on final instar of *S. litura* recorded LC_{50} values as 1165.2 and 4241.68 IJs/larva for *S. glaseri* and *H.minutus*, repectively (Prabhuraj *et al.*, 2002). Rajkumar *et al.* (2002) conducted sand column bioassay tests of *Heterorhabditis* sp. (HUDP-1 strain) against *S. litura* and recorded mortality per cent ranging from 16.7 to 88.9 when treated with 25, 50, 75, 100, 125 and 150 IJs/caterpillar. Kumar *et al.* (2003) conducted a bioefficacy test of *Heterorhabditis* sp. and recorded insect mortality

ranging from 16.6 to 88.8 per cent. 50 per cent mortality was observed within 72 h of exposure at 100IJs/100 g of soil.

In a bioefficacy experiment, *S. carpocapsae* caused 20, 25 and 50 per cent larval mortality when tested as food dip at 50,100 and 200 IJs against *S. litura*, respectively. *S. carpocapsae* when applied as foliar spray (50-100IJs/larva), food dip (25-75IJs/larva) and paper wrapping method (25-75IJs/larva) caused 40-60, 40-80 and 100 per cent mortality respectively against third instar larvae *S. litura* (Gupta, 2003). Sitaramaiah *et al.* (2003) reported that *S. carpocapsae* when applied at the dose of 4 lakh IJs m^{-2} effectively controlled *S. litura* in tobacco nursery yielding 67 per cent mortality within two days after application. Umamaheswari *et al.* (2004) studied the virulence of native strains of EPNs against *S. litura* and concluded that *H. indica* (TNAU-EPN-Hi-3) was found to be highly virulent with a LC_{50} of 3.53 IJ/larvae causing 50 per cent mortality of *S. litura* larvae in 34.52 h.

Abdel-Razek and Abd-elgawad (2007) studied the pathogenicity of seven EPN strains against the final instar larvae of cotton leaf worm, *Spodoptera litttoralis* Boisduval in a petri dish assay after exposing it to a dose of 100 IJs ml^{-1} and found that *Heterorhabditis* sp. ELG., *H. indica*, and *Heterorhabditis* sp. ELB gave cent per cent mortality of larvae within 24 h of treatment. A soil bioassay was conducted and it was found that *H. indica* had a quicker mortality rate with lower LT_{50} (32-81 h) compared to *S. carpocapsae* (33-87 h) against third instar *S. litura* when exposed to a dose of 5 to 35 IJs/larva. The experiment also reported a lower LC_{50} value of *H. indica* than that of *S.carpocapsae* in 10 per cent moisture of sand, red and black soils after 48 h of treatment in third instar larvae of *S. litura* (Raveendranath *et al.*, 2007).

Gupta *et al.* (2008) reported 100 per cent mortlity of 3-5 instar larvae of *S. litura* after 96 h of treatment in laboratory conditions by a local isolate of *S. carpocapsae*. Fifth instar larvae of *S. litura* showed maximum multiplication of

$3.29x10^5$ IJs/larvae when treated with 160 IJs/larva and LC50 values ranging from 11.41-27.17 IJs/larva when considering all the instars. Pervez and Ali (2009) observed that *in vivo* production on *S. litura* showed the maximum yield of *S. mushtaqi* ($0.79x10^5$ IJs/cadaver) followed by *S. seemae* ($0.72x10^5$ IJs/cadaver), *S. carpocapsae* ($0.67x10^5$ IJs/cadaver) and *S. masoodi* ($0.51x10^5$ IJs/cadaver).

Adiroubane *et al.* (2010) tested the efficacy of *Steinernema siamkayai* against 3rd, 4th and pre pupal instar of *S. litura* and reported that with increase in the concentration of the IJs the susceptibility of larvae also increased. A bioefficacy test evaluated under laboratory conditions using *H. indica* and *S. carpocapse* found that the 3rd and 4th instar larvae of *S. litura* when exposed to 2, 5, 10, 20 and 40 IJs cm^{-2} recorded highest mortality of 4th instar larvae than 3rd instar lavae. *H. indica* was found to be the best causing significantly higher mortality than *S. carpocapse* at lower IJs level. The level of IJs didn't affect the reproductive potential of nematode (Holajjer *et al.*, 2014).

2.4.2.4 *Gram Pod Borer, Helicoverpa armigera Hübner*

Prabhuraj *et al.* (2006) found that there was cent per cent mortality of third instar larvae @ 100 IJs/larva and 94.7 per cent mortality in case of fourth instar inferring that third instar larvae of *H. armigera* are more susceptible than fourth instar.

Saravanapriya and Subramanian (2007) reported an LC_{50} value of 10.51 IJs and 104.45 IJs of *H. indica* against larvae and pupae of *H. armigera* respectively. LC_{50} value of 122.73 IJs was observed for pupal stages of *H. armigera* by *S. glaseri*. In a filter paper assay *H. armigera* was found highly susceptible to *H. indica* causing 73–100 per cent mortality within 48 h after treatment with a LC_{50} value of 290 IJs/larva (Prasad *et al.*, 2012).

Ali and Ahmad (2009) conducted a sand assay test in pot and field conditions and observed that the maximum mortality of soil dwelling stage (pupa) of *H. armigera* was recorded in a dose of 200-500 IJs of *S. masoodi* in 100 g of soil. The adult emergence from pupa was observed as 15-25 per cent as compared to 95 per cent in control. In field condition, when a dose of 6 $x10^9$ IJs of *S. masoodi* applied in a plot of 50x50 cm recorderd 12 per cent adult emergence against 92 per cent in control. In a laboratory experiment for evaluating the pathogenicity of *H. indica* against larvae of *H. armigera*, 15, 20, 28 and 40 per cent mortality of 2^{nd} , 3^{rd} , 4^{th} and final instar larvae of *H. armigera* was observed when exposed for 24 h and treated at 300 IJs/larva (Divya *et al.*, 2010).

2.4.2.5 *Yellow Stem Borer, Scirphophaga incertulus Walker*

Rao *et al.* (1971) found that there was reduction in rice yellow stem borer, *S. incertulas* incidence on spraying of *Steinernema* sp. (DD-136) or its application in standing water. Katti *et al.* (2003) found that topical application of *Oscheius* sp. at 40 IJs/egg mass isolated from vertisols of Hyderabad was infecting egg masses of rice yellow stem borer. It prevented hatching up to 16 per cent of the eggs in an egg mass and mortality of the infected larvae ranged from 11 to 98 per cent.

A work conducted by Padmakumari *et al.* (2007) reported that *Rhabditis* sp. caused mortality of egg mass and neonate larvae of rice yellow stem borer at 500 IJs/larvae at 31 h. The EPN was found more effective during dry season than in wet season.

2.4.3 Dipteran

Rinker *et al.* (1995) found *S. feltiae* and *H. heliothidis* causing 38 per cent and 52 per cent mortality respectively of mushroom sciarid, *Lycoriella mali* Fitch. *S. feltiae* when applied at 30 IJ per larvae caused 78 per cent mortality of mushroom fly, *Megaselia haltera* (Scheepmaker *et al.*, 1998).

Gazit *et al.* (2000) evaluated 12 EPN strains against the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann and found that *S. riobrave* Texas (Sr TX) and *Heterorhabditis* sp. IS-5 (H IS-5) showed the highest mortality of more than 80 per cent whereas six EPN strains showed less than 30 per cent mortality and four strains showed less than 20 per cent mortality. EPN infectivity was directly related to nematode density with the maximum activity shown at a density of 150 IJs cm^{-2}. The EPNs remained virulent in the soil for about 5 days but there was no activity after 14 days.

Mortality of *Bactrocera cucurbitae* Coq. larvae ranged from 6- 47 per cent at an inoculum level of 50, 100 and 200 IJs per larvae by *Rhabditis* sp. They also reported 87 and 100 per cent mortality at 48 and 72 h after treatment respectively when treated with 200 IJs per larvae (Sheela *et al.*, 2002). Hussein *et al.* (2006) conducted a filter paper assay and reported that *S. feltiae* caused 50 per cent mortality of 2nd instar larvae of *Dacus ciliatus* Loew at 100 IJs/larvae and recorded 90 per cent mortality at 500 IJs/larvae. At the 7th day of application of EPN, mortality reached 100 per cent for both the two concentrations.

Jacob and Mathew (2016) studied the effectiveness of entomopathogenic nematodes isolated from soils of Kerala in causing mortality to *Liriomyza trifolii* Burgess maggots inside the mines. They reported that *S. carpocapsae* Isolate 1 (Kannara) was found to be more effective against *L. trifolii* larvae with lowest LC_{50} value (1.79/ maggot).

2.4.4 Hemipterans

Sandner and Pezowicz (1983) conducted laboratory experiments in Poland against cabbage aphid, *Brevicoryne brassicae* Linnaeus and reported 100 per cent mortality after 48 h when treated with 10 IJs $insect^{-1}$.

Brown *et al.* (1992) conducted field trials against *Eriosoma lanigerum* Hausmann, in an unsprayed 6 and 4 year old apple orchards in West Virginia to test

the efficacy of broadcast spray and topdressing applications of nematodes at 3, 76,600 IJs m^{-2} and found that the broadcast spray trees had fewer aphid colonies on roots than the untreated controls, but the topdressing treatment had no effect.

Farag (2002) evaluated the pathogenicity of two native Egyptian isolates, *H. taysearеae* and *S. carpocapsae* S2, against *Aphis fabae* Scopoli and found that *A. fabae* was highly susceptible in laboratory conditions and a single spray of 1000 IJs ml^{-1} of either *H. taysearae* or *S. carpocapsae* S2 provided a mortality of 58.33 per cent, after one week of application when applied to soyabean plants infested with *A. fabae* under greenhouse conditions.

Mohandas *et al.* (2004) isolated a pathogenic species *Rhabditis* (*Oscheius*) sp. which was found to be effective against arecanut spindle bug, *Carvalhoia arecae* Miller and China.

A laboratory experiment was carried out using five native isolates and standard culture, *H. indica* and *S. glaseri* at 10, 25, 50 and 100 IJs/rice bug. Isolate 5 proved the best with cent per cent mortality at lowest inoculum level of 10 IJ (Sheela *et al.*, 2006).

Kumar and Ganguly (2011) conducted a leaf disc assay and reported that *S. thermophilum* caused 83.00 per cent mortality at 50 IJs ml^{-1} within 72 h after inoculation and 100 per cent at 500 IJs ml^{-1} within 48 h against third instar nymphs of solenopsis mealybug, *Phenacoccus solenopsis* Tinsley. In case of *Aphis gossypi* Glover, *S. thermophilum* caused 66 and 83 per cent mortality @ 50 and 500 IJs ml^{-1}, respectively after 3 days of treatment. Maketon *et al.* (2011) reported that *H. indica* was able to penetrate and kill *A. gossypii* but reproduced poorly.

2.4.5 Termites

Nancy and John (1988) found that termite workers of *Reticulitermes flavipes* Kollar were susceptible to *S. feltiae* in laboratory tests, but large numbers of nematodes were required for mortality. LD_{50} was estimated as 1.5×10^4 IJs per termite in standard filter paper assays.

Zadji *et al.* (2014) reported that workers of *Macrotermes bellicosus* Smeathman was highly susceptible to 50 IJs of *H. indica*, *H. sonorensis*, *H. sonorensis* and *Steinernema* sp. resulting in 96.3, 87.9, 94.5 and 75.0 per cent mortality respectively whereas these EPN isolates caused 91.7, 98.5, 75.0 and 95.0 per cent mortality of workers of *Trinervitermes occidentalis* Snyder under the same conditions.

Razia and Sivaramakrishnan (2016) conducted a sand assay test and found that *S. pakistanense* showed cent percent mortality of both *R. flavipes and Odontotermis hornei* within 24 h of treatment than *S. siamkayai. H. indica* caused cent per cent mortality at 48h with 250 IJs ml^{-1}. *H. indica* required less number (5IJs/termites) and less time (20 h) to cause significant mortality against *R. flavipes. S. pakistanense* was found to produce more offspring within termites.

Wagutu *et al.* (2017) reported cent per cent mortality of *Coptotermes formosanus* with 100 IJs/ termite of *S. karii* at 96 HAT.

Materials and Methods

3. MATERIALS AND METHODS

The study was aimed to isolate indigenous entomopathogenic nematodes (EPNs) from the fields of vegetables, banana and coconut of Thiruvanathapuram, Kollam, Pathanamthitta and Alappuzha districts of Kerala by soil sampling and to determine their potential in causing mortality to important pests of horticultural crops. The isolates collected were identified through taxonomic keys and further confirmed by molecular characterization.

3.1 ISOLATION OF INDIGENOUS EPNs

A random sampling of EPN isolates was conducted in four districts of Kerala and they were screened against test insects. Taxonomic identification was carried out at the Department of Nematology, College of Agriculture, Vellayani, Kerala. A total of forty soil samples were collected from rhizosphere region of different crop habitats. The soil samples were collected from fields having extensive cultivation of the respective crops.

3.1.1 Sampling

Soil samples and dead cadavers were collected from the rhizosphere of above mentioned crops. Top soil from the base of the plant was removed and around 500 cc soil samples were collected from a depth of 10-30 cm. The collected samples were put in polythene bags of 150 gauge thickness, tied with a rubber band. Information regarding date of sampling, standing crop in the field and soil type along with GPS (Global Positioning System) location was recorded. Samples were maintained in refrigerated conditions for further processing. The soil was thoroughly mixed and 250cc of each sample was used for extraction of EPNs.

3.1.2 Rearing of Trap Insect, Rice Moth (*Corcyra cephalonica* Stainton)

Corcyra cephalonica was reared in the laboratory of Department of Nematology on diet prepared with crushed maize. The crushed maize was sterilized in an oven at 100°C for 30 minutes in the laboratory. Broken groundnut kernel (100 g) was transferred to each basin and the contents were hand mixed. Dry yeast and wettable sulfur was added @ 5g per basin and were mixed thoroughly. Two hundred numbers of 3rd instar *C. cephalonica* larvae were collected from Biocontrol laboratory at Parottukonam and were released in 2.5 kg of feed in plastic containers and covered with a piece of muslin cloth (Plate 1). The plastic containers were kept in room temperature in the laboratory. The moths were collected and transferred to separate oviposition containers for egg laying. The eggs collected from oviposition containers were put in fresh artificial diet for maintaining the culture of *C. cephalonica* larvae.

3.1.3 Isolation of Indigenous EPN

The larvae of *C.cephalonica* were used to concentrate EPN from the soil samples. Fifth instar larvae reared in standard medium was used for trapping EPN as described by Woodring and Kaya (1988).

3.1.3.1 *Insect Baiting Technique*

EPNs were isolated from the soil samples by the method described by Bedding and Akhurst (1975) with larvae of *C. cephalonica*. The soil samples were homogenized before they were baited. Ten fifth instar larvae of *C. cephalonica* were released into the plastic container containing 200 g of soil sample. Baited samples were stored in dark at room temperature (26±2°C) (Plate 2). Samples were monitored for mortality upto 12 days. Insect cadavers from each soil sample were taken out and examined for infection.

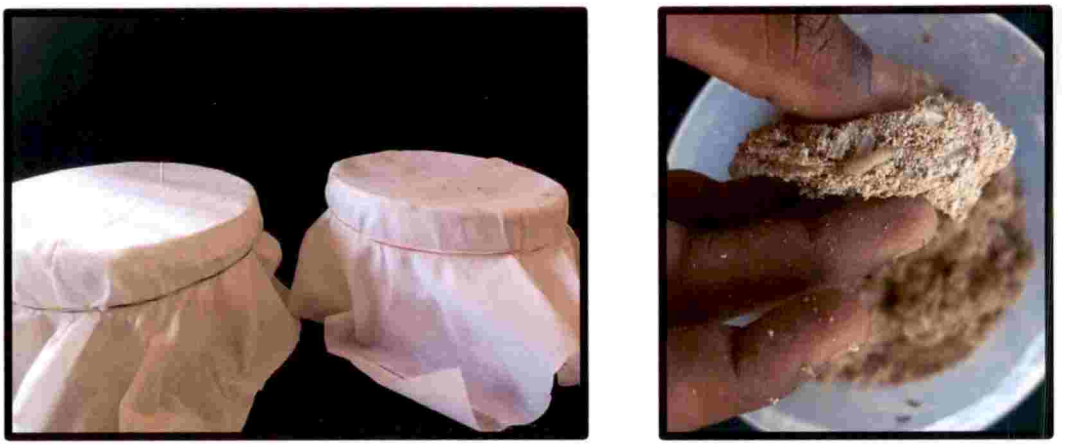


Plate 1. Rearing of rice moth larvae

Plate 2. Baiting of soil samples

3.1.3.2 *Extraction of Infective Juveniles (White trap technique)*

Fifth day after baiting, soil from the plastic containers were emptied into a pan and observed the larvae for infection by the EPNs. Dead larvae were collected from the container and was surface-sterilized in 1.0 % sodium hypochlorite solution for 3 minutes, then washed three times in sterile distilled water (Chandler *et al.*, 1997) and placed in "White trap" (White, 1927). To make "White trap" an inverted watch glass was placed in a sterilized petri dish. Sterile distilled water, 50 ml, was poured into the petri dish. A Whatman No. 1 filter paper was placed over the watch glass so that it comes in contact with the liquid surface. Infected dead larvae were placed on the filter paper over the edge of the watch glass (Plate 3A). Infective juveniles (IJs) of EPN started to emerge from sixth to tenth day after infection (Plate 3B). The emerged IJs were collected in tissue culture flasks containing 0.1 per cent formaldehyde and kept in BOD incubator at 15°C.

3.2 SCREENING OF EPN UNDER *IN VITRO* CONDITIONS

3.2.1 Test Insects

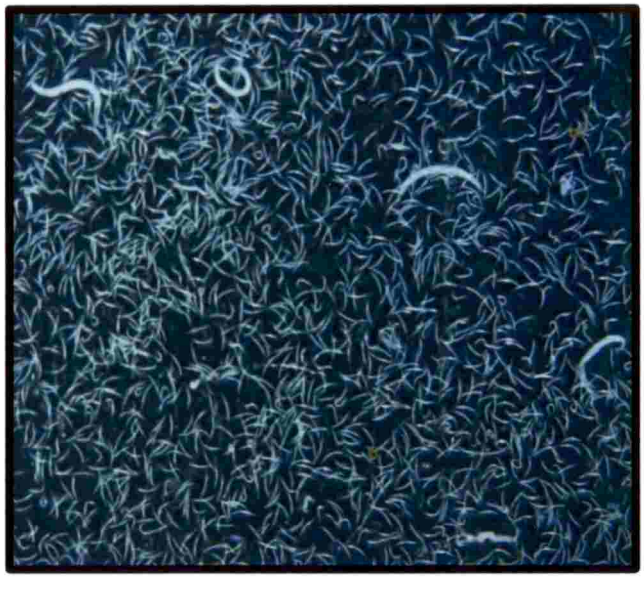
Termites, cowpea aphids, tobacco caterpillar and banana pseudostem weevil were used as the target pests.

Design: CRD

3.2.1.1 *Termites, Odontotermes obesus*

Termites were collected from the termatarium and were maintained in a container at room temperature (25-30 °C) in the laboratory (Plate 4A). Virulence of EPN isolates were studied in sand assay method where 10g of dry sand was uniformly spread in each petri plate lined with moist filter paper and 300 IJs/adult was applied to each petri dish. Fifty worker termites were introduced into the petri dish (Plate 4B). Termite mortality was recorded at 24 hours after treatment (HAT).

A. White trap used for extraction of IJs



B. Suspension of IJs (100X)

Plate 3. Extraction of native EPN stains

Dead termites were placed in the white trap for IJs extraction. Nematodes that emerged from the dead termites were collected. Colour change in termites were recorded at 72 HAT and number of IJs emerged out of each termite was counted under stereozoom microscope. The treatment was replicated five times. Sterile water was used as control.

3.2.1.2 *Aphids, Aphis craccivora*

Cowpea plants were raised in the glass house of Department of Nematology and aphids (*Aphis craccivora*) were reared in these plants. Twigs of cowpea plant infested with heavy population of aphids were collected and kept in healthy plants maintained in the glass house (Plate 5A). Preceding the experiment, non alate adults were collected and 50 numbers were brushed out into the petri dish lined with moist filter paper. Nematode suspension containing 300 IJs/aphid was inoculated into each petridish (Plate 5B). The treatment was replicated five times along with sterile water as control. Cowpea leaves were provided as food source in the petri dish. The experiment was repeated for all the native EPNs isolated. Number of dead aphids was recorded at 24 HAT and was placed in white trap for extraction of IJs. Colour change in aphids was recorded. Nematodes that emerged from the dead aphids were collected. Number of IJs emerged out of each aphid at 72 HAT was also counted under stereozoom microscope using a counting dish.

3.2.1.3 *Tobacco Caterpillar, Spodoptera litura*

Egg masses of *S. litura* were collected from field and were kept in a plastic trough which was maintained inside an insect cage for 3- 5 days. Hatched larvae were separated into a clean trough containing banana leaves and were covered with a piece of muslin cloth. Third instar larvae were transferred to the petridish lined with moist filter paper. Nematode suspension containing a concentration of 300 IJs/larva was inoculated into each petridish. The treatment was replicated five times along with a control in which sterile water was used. Banana leaves were provided as a food

A. Termatarium

B. Experimental set up

Plate 4. Pathogenicity study of native EPNs against termites

A. Aphid infested cowpea plant B. Experimental setup

Plate 5. Pathogenicity study of native EPNs against aphids

Plate 6. Pathogenicity study of native EPNs against tobacco caterpillar

source in the petri dish (Plate 6). The experiment was repeated for all the native EPNs isolated. Number of dead larvae was recorded at 24 HAT and was placed in the white trap for extraction of IJs. Colour change in cadaver was recorded. Nematodes that emerged from the cadavers were collected. Number of IJs emerged out of each cadaver at 72 HAT was also counted under stereozoom microscope using a counting dish.

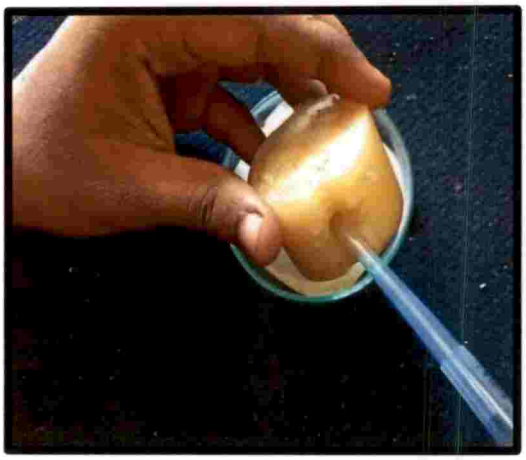
3.2.1.4 *Banana Pseudostem Weevil, Odoiporous longicollis*

Fourth instar grubs of pseudostem weevil were collected from infested banana plants (Plate 7A). Fresh 5 cm long pseudostem was taken for the experiment. Nematode suspension with a concentration of 300 IJs per grub was inoculated into already made incision in the pseudostem (Plate 7B). Five 4^{th} instar grubs of pseudostem weevil were then introduced into treated pseudostem and kept undisturbed (Plate 7C&D). The treatment was replicated five times. The pseudostem kept as control was inoculated with sterile water. The experiment was repeated for all the native EPNs isolated. Number of dead grubs was recorded at 24 HAT and was placed in the white trap for extraction of IJs. Colour change in cadaver was recorded. Nematodes that emerged from the cadavers were collected. Number of IJs emerged out of each cadaver at 72 HAT was also counted under stereozoom microscope using a counting dish.

3.3 PATHOGENICITY OF EPNS

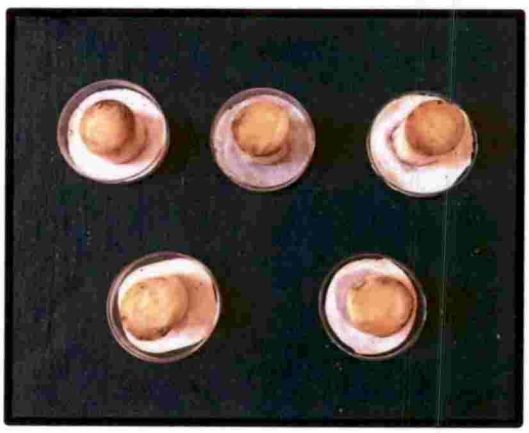
The indigenous isolates were evaluated for the pathogenicity against the test insects (termite, aphid, tobacco caterpillar and pseudostem weevil grubs) in the laboratory and their effective doses were determined. The test insects were treated with different concentration of IJs (10, 50, 100 and 200 IJs/insect) and were incubated at room temperature. Each concentration of indigenous isolates was replicated four times. Mortality of the insects was recorded at 24, 36, 48, 60 and 72

A. Pseudostem weevil grubs



B. Inoculation of IJs

C. Release of grubs



D. Experimental setup

Plate 7. Pathogenicity study of native EPNs against pseudostem weevil grubs

HAT. The corrected mortality percentage was worked out by using Abbott's formula (Abbott, 1925) and compared with that of standard chemical.

$$\text{Corrected mortality (Abbott, 1925)} = \frac{[T-C]}{100-C} \times 100$$

Where T- mortality in treatments C -mortality in control

3.3.1 Termites

Design- CRD

Treatments- 10, 50, 100, 200 IJs of three indigenous isolates, sterile water and chemical check

Replication- Four

The concentration *viz.*, 10, 50, 100 and 200 IJs of three indigenous isolates were taken from the stock culture. Termite culture was maintained as mentioned in 3.2.1.1 and fifty termites were released into the petri plate. Chlorpyriphos 20 EC 0.2 % was used in chemical check for the experiment. Mortality was noted at 24, 36, 48, 60 and 72 HAT. The cadavers were transferred to white trap apparatus and the emerging IJs were collected (Plate 8A).

3.3.2 Aphids

Design- CRD

Treatments- 10, 50, 100, 200 IJs of three indigenous isolates, sterile water and chemical check

Replication- Four

The concentration *viz.*, 10, 50, 100 and 200 IJs of three indigenous isolates were taken from the stock culture. As mentioned in 3.2.1.2 the experimental set up

was made and fifty non-alate aphids were transferred into the petri plate. Dimethoate 30 EC 0.2 % was used as chemical check for the experiment. Mortality was noted at 24, 36, 48, 60 and 72 HAT. The cadavers were transferred to white trap and the emerging IJs were collected (Plate 8B).

3.3.3 Tobacco Caterpillar

Design- CRD

Treatments- 10, 50, 100, 200 IJs of three indigenous isolates, sterile water and chemical check

Replication- Four

The concentration *viz.*, 10, 50, 100 and 200 IJs of three indigenous isolates were taken from the stock culture. As mentioned in 3.2.1.3 the experimental set up was made and ten larvae were released into the petri plate. Flubendiamide 39.35 SC 0.01 % was the chemical check for the experiment. Mortality was noted at 24, 36, 48, 60 and 72 HAT. The cadavers were transferred to white trap and the emerging IJs were collected (Plate 8C).

3.3.4 Pseudostem Weevil

Design- CRD

Treatments- 10, 50, 100, 200 IJs of three indigenous isolates, sterile water and chemical check

Replication- Four

The concentration *viz.*, 10, 50, 100 and 200 IJs of three indigenous isolates were taken from the stock culture. As mentioned in 3.2.1.4 the experimental set up was made and ten pseudostem weevil grubs were released into the pseudostem. Chlorpyriphos 20 EC 0.2 % was the chemical check for the experiment. Mortality

was made and fifty non-alate aphids were transferred into the petri plate. Dimethoate 30 EC 0.2 % was used as chemical check for the experiment. Mortality was noted at 24, 36, 48, 60 and 72 HAT. The cadavers were transferred to white trap and the emerging IJs were collected (Plate 8B).

3.3.3 Tobacco Caterpillar

Design- CRD

Treatments- 10, 50, 100, 200 IJs of three indigenous isolates, sterile water and chemical check

Replication- Four

The concentration *viz.*, 10, 50, 100 and 200 IJs of three indigenous isolates were taken from the stock culture. As mentioned in 3.2.1.3 the experimental set up was made and ten larvae were released into the petri plate. Flubendiamide 39.35 SC 0.01 % was the chemical check for the experiment. Mortality was noted at 24, 36, 48, 60 and 72 HAT. The cadavers were transferred to white trap and the emerging IJs were collected (Plate 8C).

3.3.4 Pseudostem Weevil

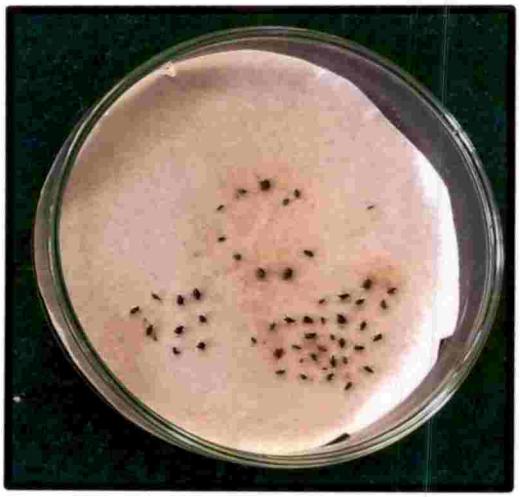
Design- CRD

Treatments- 10, 50, 100, 200 IJs of three indigenous isolates, sterile water and chemical check

Replication- Four

The concentration *viz.*, 10, 50, 100 and 200 IJs of three indigenous isolates were taken from the stock culture. As mentioned in 3.2.1.4 the experimental set up was made and ten pseudostem weevil grubs were released into the pseudostem. Chlorpyriphos 20 EC 0.2 % was the chemical check for the experiment. Mortality

A. Termites



B. Aphids

C. Tobacco caterpillar

D. Pseudostem weevil

Plate 8. White trap of test insects

3.5.3 Light Microscopic Studies

Morphological characters of 15 specimens each of IJs, males and females were observed using ZEISS microscope under 10, 40, 100 and 200X magnification. The permanent slides were examined for detailed morphological characters and body dimensions were studied using de Man's formula (de Man, 1880) and ratios were calculated to establish their taxonomic identity. The morphological identification was performed on the basis of characters of third stage IJs, hermaphrodites, females and male individuals (Stock, 2002).

3.5.3.1 *Morphological Characters*

The following morphological characters were taken into consideration for identification at species level.

(a) Shape of head

(b) Shape and size of spicules

(c) Shape and size of gubernaculum

(d) Presence or absence of post anal swelling in adult females

(e) Tail shapes of both adults and IJs

(f) Presence or absence of mucron in adults of both sexes

3.5.3.2 *Morphometric Measurements*

3.5.3.2a *Linear Body Dimensions Recorded were as Follows*

1. Body length (L)
2. Body width (W)
3. Oesophageal length (ES)
4. Distance from anterior end to excretory pore (EP)
5. Spicule length (SL)
6. Gubernaculum length (GL)

7. Anal body width (ABW)
8. Tail length (T)

3.5.3.2b *The Following Ratios were Computed*

1. a = Body length/ Greatest body width
2. b = Body length/ Oesophageal length
3. c = Body length/ Tail length
4. V = Distance of vulva from anterior end/ Body length×100
5. D% = Distance from anterior end to excretory pore / Oesophageal length×100
6. E%= Distance from anterior end to excretory pore/ Tail length×100
7. F% = Maximum body length/ Tail length×100
8. SW% = Spicule length/ anal body width×100
9. GS% = Gubernaculum length/ Spicule length×100

3.6 MOLECULAR CHARACTERIZATION OF POTENTIAL EPN STRAIN

3.6.1 DNA Extraction

The procedure was performed as described by Miller *et al.* (1988) for DNA extraction. About 2000-3000 sterilized IJs were used for DNA extraction.

3.6.1.1 *Solutions Required For DNA Extraction and Gel-electrophoresis*

a. *DNA Extraction*

1. DNA extraction buffer - 50mM Tris HCl (pH- 0.8), 400mM NaCl, 20mM EDTA (pH- 0.8), 0.5% SDS
2. Proteinase K
3. 5M NaCl
4. Absolute ethanol
5. 70 % ethanol
6. TE Buffer – 10mM Tris HCl, 1mM EDTA

b. *Gel-electrophoresis*

1. 50X Tris-acetate (TAE)
2. Trisbase- 121 g

3. Glacial acetic acid- 28.55 ml
4. 0.5 M EDTA (pH 8.0)- 50 ml

c. *Ethidium Bromide*

Ethidium bromide, 100 mg, was mixed with 10 ml of sterilized water and was stirred on a magnetic stirrer till the dye gets completely dissolved. The container was covered with aluminum foil and stored in room temperature.

d. *6X Loading Dye*

Bromophenol blue 0.25, xylene cyanol 0.25% and glycerol 30% in water which are kept at 4°C.

3.6.1.2 *The Procedure for DNA Extraction of EPN Species (Singh, 2009)*

a. *Tissue Digestion*

IJs were crushed in 600µl of TNES solution (50mM Tris HCl, 400mM NaCl, 20mM EDTA, 0.5% SDS) by using polypropylene pestle which is shaped to fit closely at the base of Eppendorf tubes. After crushing, Proteinase K (10µl) was added. The crushed IJs were gently vortexed and the tube was incubated at 65°C for 3 h with occasional vortexing.

b. *Precipitation of Proteins and Cell Debris*

To precipitate out the proteins, the tubes were removed after 3 h and 170 µL of 5M NaCl was added. The suspension in the tubes was mixed thoroughly by shaking the tubes up and down for 15 s. The tubes were centrifuged at 14000 rpm for five minutes. While placing the tubes into the centrifuge the lid hinge of the tubes were pointed away from the center to ensure that the pellets were formed on one side of each tube. The supernatant containing the DNA was pipetted into clean new labeled tubes carefully taking precautions that the cell and protein debris did not contaminate the DNA.

c. *Precipitation of Nucleic Acids*

The supernatant containing DNA was added to 750 µL of cold absolute ethanol and they were mixed thoroughly by inverting the tubes gently for 30 s to precipitate out the DNA. After precipitation of DNA, the tubes were centrifuged at

14000 rpm for five minutes. The ethanol and the salts were poured off without disturbing the DNA pellet formed after centrifugation. The DNA pellet was rinsed by adding 400 µL of 70% ethanol and centrifuging at 14000 rpm for 5 min. The ethanol was poured off and the remaining ethanol was allowed to evaporate by leaving the tubes inverted for 20-30 min on top of clean paper towel. The DNA pellet was suspended in 30 µL of TE buffer (10mM Tris HCl, 1mM EDTA) and stored at -200^{0}C for later use in PCR.

d. *Agarose Gel-electrophoresis*

The DNA was electrophoresed on 0.8% agarose gel at 80V - 100V and 70 mA for two hours in TAE buffer. The gel was stained using ethidium bromide (0.5µg/ml) and was observed on UV-transilluminator.

3.6.2 Molecular Marker Studies

3.6.2.1 *Standardization of Polymerase Chain Reaction (PCR) Procedure*

Polymerase Chain Reaction (PCR) protocol was standardized for carrying out amplification using species specific primers. Reaction conditions and optimum concentration of various components *viz.*, template DNA, $MgCl_2$, dNTPs, Taq DNA polymerase and primers were standardized. The following reagents were used for carrying out the procedure:

10X buffer	-	5 µl
dNTP mix	-	2 µl
$MgCl_2$	-	4 µl
18S (forward primer)	-	2.5 µl
18S (Reverse primer)	-	2.5 µl
Taq polymerase	-	2 µl
Template DNA	-	2 µl
Nuclease free water	-	30 µl

Different quantities of each reaction component were taken to prepare the reaction volume. The volume was completed using sterile distilled water. The reagents were thoroughly mixed in an eppendorf tube of 1.5 ml capacity and vortexed for 15 s. This mixture was evenly distributed to each 0.2 ml PCR reaction tube and then DNA (2.0 µl) was added separately in each tube. Cyclic amplification of the tubes was done in a thermocycler.

The DNA samples from different genotypes of EPNs were amplified using PCR using the protocol of Williams *et al.* (1990). DNA amplification was carried out in a thermocycler. The steps of DNA amplification, temperature requirement and time period is mentioned below:

Sl. No.	Step	Temperature (°C)	Time (min)
1	Initial denaturation	95	4
2	Denaturation	94	1
3	Annealing	60	1
4	Extension	72	2
5	Final Extension	72	8

Total number of cycle- 35

Further, the amplified products were stored at 4°C for electrophoresis.

3.6.2.2 *Electrophoresis of Amplified DNA*

The amplified DNA was thoroughly mixed with 6X loading dye and then electrophoresed in 1.4% agarose gel in 1X TAE buffer. The gel was run at constant voltage at the rate of 5V/cm under submerged conditions for about 3 h. 0.5 µg/ml ethidium bromide was incorporated in the gel. The size of the amplified product was determined by co-electrophoresis of 100 bp standard molecular weight marker. DNA profile were visualized on a UV transilluminator and photographed by using Gel Documentation System.

3.7 DNA SEQUENCING

Molecular characterization of the most potent isolate was done by amplifying the internal transcribed region using TTGATTACGTCCCTGCCCTTT as forward primer and TTTCACTCGCCGTTACTAAGG as reverse primer (Vrain *et al.*, 1992). Purified PCR products were sequenced using the DNA sequencing servicer provided by the company SciGenom. The DNA sequence analysis of the 18s rRNA of the nematode isolate was done in NCBI website using BLAST.

3.8 STATISTICAL ANALYSIS

The data generated from the experiments 4.2 to 4.3 were subjected to analysis of variance (ANOVA) technique (Cochran and Cox, 1965). The variables which did not satisfy the basic assumptions of ANOVA were subjected to angular transformations and analysed.

Results

4. RESULTS

4.1. ISOLATION OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES

A total of forty samples were collected from the rhizosphere of vegetables, banana and coconut grown in Thiruvanathapuram, Kollam, Pathanamthitta and Alappuzha districts by random sampling. Three isolates of EPN were isolates by using insect baiting technique and were grouped into three categories Isolate 1, 2 and 3. Isolate 1 was obtained from the sample collected from cowpea plant grown in College of Agriculture, Vellayani, Thiruvanathapuram. Isolate 2 was obtained from the sample collected from tomato plant grown in a multicropped field in Mylom, Kottarakara (Kollam). Isolate 3 was obtained from the banana rhizosphere in Kainidi area of Alappuzha district. The frequency of distribution of EPNs in Thiruvananthapuram, Kollam and Alappuzha was recorded as 10 per cent (Table 1). No EPN species were obtained from the samples collected from Pathanamthitta district.

4.2. SCREENING OF EPN ISOLATES FOR INSECTICIDAL PROPERTY UNDER *IN VITRO* CONDITIONS

4.2.1 Termites

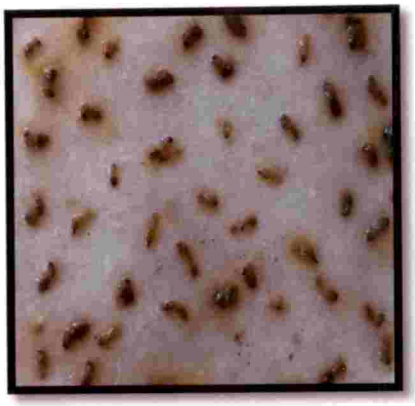
Among the three native strains, Isolate 2 caused the highest mortality (87.99 per cent) followed by Isolate 1 (86.00 per cent) and Isolate 3 (76.00 per cent) at 24 HAT with 300 IJs/termite. Cadavers infected with Isolate 2 were brownish in colour whereas Isolates 1 and 3 showed no appreciable colour change (Plate 9). The emergence of IJs per insect was found highest in Isolate 2 (2.0×10^3) at 72 HAT (Plate 10). Isolate 1 and 3 showed an emergence of 1.4×10^3 IJs and 0.6×10^3 IJs respectively (Table 2).

Table 1: Distribution of indigenous isolates of entomopathogenic nematodes in different districts of Kerala

District	Place of collection	GPS Co-ordinates	Name of the crop	Number of Native isolate obtained	Percentage frequency of occurrence
Thiruvananthapuram	COA, Vellayani Poonkulam	8.4410 76.9891	Cowpea	1	10
Kollam	Mylom, Kottarakara	8.5490 76.3877	Tomato	1	10
Pathanamthitta	-	-	-	0	0
Alappuzha	Kainadi, Kavalam	9.4925 76.4703	Banana	1	10

n (Number of samples collected) = 10

Isolate 1



Isolate 2

Isolate 3

Plate 9. Colour change in termites due to native EPN strains

Plate 10. Emergence of EPN from termite cadaver

4.2.2 Aphids

Isolate 2 recorded the highest mortality of aphids (86.00 per cent) followed by Isolate 1 (81.74 per cent) and Isolate 3 (71.99 per cent) with 300 IJs/aphids at 24 HAT. No appreciable colour change was observed in the cadavers infected with any of the isolates (Plate 11). Isolate 2 showed the highest emergence of IJs per insect ($1.2.x10^{3}$) compared to Isolate 1 ($0.9x10^{3}$) and 3 ($0.2x10^{3}$) (Plate 12) (Table 2).

4.2.3 Tobacco Caterpillar

Highest mortality of tobacco caterpillar was observed with Isolate 2 (29.99 per cent) followed by Isolate 1(19.99 per cent) and Isolate 3 (19.99 per cent) with 300 IJs per larvae at 24 HAT. The cadavers infected with Isolate 2 had pinkish colour whereas cadavers infected with Isolate 1 and 3 did not show any colour change (Plate 13). Isolate 2 showed the highest emergence of IJs per insect ($3.5x10^{5}$) compared to Isolate 1 ($2.7x10^{5}$) and 3 ($0.9x10^{5}$) (Plate 14) (Table 3).

4.2.4 Pseudostem weevil

Among the three native strains, Isolate 1 and 2 at 300 IJs/ pseudostem weevil grub showed a mortality percentage of 9.99, whereas no mortality was observed in Isolate 3 at 24 HAT. Isolate 1 and 2 showed a discolouration of creamish and reddish brown in cadavers respectively, whereas Isolate 3 did not show any colour change in cadavers (Plate 15).The emergence of IJs per insect was found highest in Isolate 2 ($3.5x10^{5}$) compared to Isolate 1 ($1.5 x10^{5}$) and 3 ($0.3x10^{5}$) (Plate 16) (Table 3).

4.3 PATHOGENICITY OF EPN

The potential of indigenous EPN isolates were assessed by inoculating the Infective Juveniles (IJs) at 10, 50, 100 and 200 against test insects (termites, aphids,

Table 2: Effect of different isolates on the mortality of termites and aphids and emergence of IJs

Isolates* (IJs)	TERMITES			APHIDS		
	% Mortality (24h)	Colour change	Emergence (IJs/test insect)	% Mortality (24h)	Colour change	Emergence (IJs/test insect)
1	86.00 (68.03)	No change	$1.4x10^{3}$	81.74 (64.70)	No change	$0.9x10^{3}$
2	87.99 (69.78)	Brownish	$2.0x10^{3}$	86.00 (68.03)	No change	$1.2.x10^{3}$
3	76.00 (60.67)	No change	$0.6x10^{3}$	71.99 (58.05)	No change	$0.2x10^{3}$
CD (0.05)	(5.32)	-	-	(4.89)	-	-

* At the rate of 300

Figures in parenthesis are arc sine transformed values

Table 3: Effect of different isolates on the mortality of tobacco caterpillar and pseudostem weevil grubs and emergence of IJs

Isolates* (IJs)	TOBACCO CATERPILLAR			PSEUDOSTEM WEEVIL		
	% Mortality (24h)	Colour change	Emergence (IJs/test insect)	% Mortality (24h)	Colour change	Emergence (IJs/test insect)
1	19.99 (26.56)	No change	$2.7x10^5$	9.99 (18.43)	Creamish	$1.5 x10^5$
2	29.99 (33.21)	Pinkish	$3.5x10^5$	9.99 (18.43)	Reddish brown	$3.5x10^5$
3	19.99 (26.56)	No change	$0.9x10^5$	0.00 (0.03)	No change	$0.3x10^5$
CD (0.05)	(4.38)	-	-	NS	-	-

* At the rate of 300

Figures in parenthesis are arc sine transformed values

Isolate 1

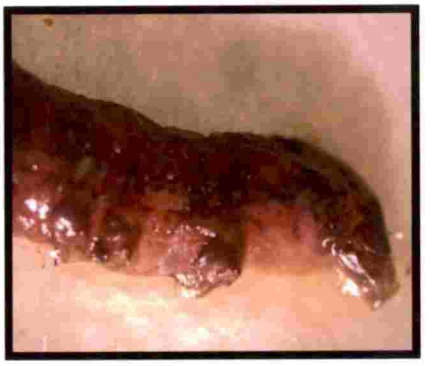
Isolate 2

Isolate 3

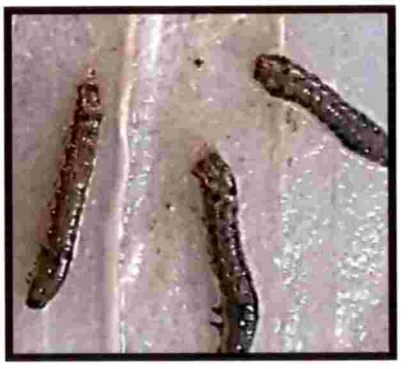
Plate 11. Colour change in aphids due to native EPN strains

Plate 12. Emergence of EPN from aphid cadaver

Isolate 1



Isolate 2



Isolate 3

Plate 13. Colour change in tobacco caterpillars due to native EPN strains

Plate 14. Emergence of EPN from tobacco caterpillar cadaver

Isolate 1

Isolate 2

Isolate 3

Plate 15. Colour change in pseudostem weevil grubs due to native EPN strains

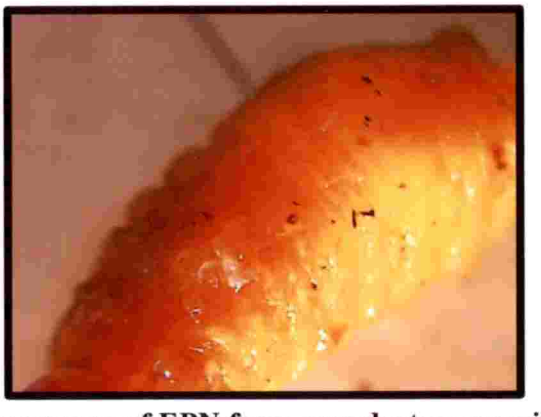


Plate 16. Emergence of EPN from pseudostem weevil cadaver

tobacco caterpillar and pseudostem weevil). Observations were recorded at 24, 36, 48, 60 and 72 h after exposure. The results are presented in Tables 4-15.

4.3.1 Isolate 1

4.3.1.1 *Termites*

The results presented in Table 4 revealed that there was statistically significant variation between different levels of IJs of Isolate 1 on mortality of termites at 24, 36, 48, 60 and 72 hours after treatment (HAT).

Among the different concentrations of IJs tested, 200 IJs showed the highest mortality with a corrected mortality percentage of 73.62 and it was significantly superior to lower concentrations of IJs *viz.* 100, 50 and 10. The corrected mortality percentage in the lower concentrations of IJs ranged from 41.98 to 68.03 and it was significantly different. Highest mortality of termites (cent per cent) was observed in chemical treatment.

Considering different concentrations of IJs of Isolate 1, the effect of 200 and 100 IJs was statistically on par with the chemical with a corrected mortality percentage of 93.37, 87.82 and 100 respectively at 36 HAT. The lower concentrations of IJs *viz.* 10 and 50 was inferior to 200 and 100 IJs with per cent mortality of 72.06 and 57.06 respectively.

Highest mortality of termites (cent per cent) was observed in 100 and 200 IJs and it was statistically on par with the chemical at 48 HAT. The corrected mortality percentage of termites observed in 50 IJs was 85.16 and it was inferior to above treatments. Isolate 1 at 10 IJ level was inferior to 50 IJs and gave a mortality of 72.63 per cent.

Isolate 1 at 50 IJ level recorded 99.08 per cent mortality of termites at 60 HAT. Effect of this treatment was statistically on par with higher inoculum levels

Table 4: Mortality of termites due to Isolate 1 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	41.98[e] (40.38)	57.06[c] (49.06)	72.63[c] (58.46)	88.10[b] (69.82)	100.00[a] (90.00)
50	55.03[d] (47.89)	72.06[b] (58.09)	85.16[b] (67.35)	99.08[a] (84.51)	100.00[a] (90.00)
100	68.03[c] (55.57)	87.82[a] (69.58)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
200	73.62[b] (59.09)	93.37[a] (75.08)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
Chlorpyriphos 25 EC (2ml/l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD (0.05)	(5.059)	(8.256)	(5.080)	(7.661)	NS

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

(100 and 200 IJs) and chemical. The lowest inoculum level of 10 IJs recorded 88.10 per cent mortality of termites which was inferior to all other treatments.

Isolate 1 at lowest inoculum levels of 10 IJs recorded cent per cent mortality of termites at 72 HAT. Effect of this treatment was statistically on par with higher inoculum levels (50, 100 and 200 IJs) and chemical check.

4.3.1.2 *Aphids*

The results presented in Table 5 showed statistically significant variation between different concentrations of IJs of Isolate 1 on mortality of aphids at 24, 36, 48, 60 and 72 HAT.

The per cent mortality of aphids at different concentrations of IJs was significantly different from the chemical (Dimethoate 30 EC) treatment. Among the different concentrations of IJs, 200 IJs showed significantly higher mortality than all concentrations of IJs. The mortality with 100 IJs was 68.52 percent and it was significantly superior to lower concentrations (10 and 50 IJs). A mortality percentage of 50.00 was recorded with 50 IJs and it was significantly superior to 10 IJs. Minimum mortality was recorded with 10 IJs and it showed a corrected mortality percentage of 38.49.

At 36 HAT, the per cent mortality obtained with different concentrations of IJs was significantly different from the chemical which showed cent per cent mortality. Among the different concentrations of IJs, 200 IJs showed the highest mortality with a corrected mortality percentage of 89.56 and it was found significantly superior to all other concentrations of IJs. The mortality of aphids treated with 100 IJs was 82.02 percent and it was significantly superior to 50 and 10 IJs which showed a mortality percentage of 63.51 and 45.99 respectively.

Among the different concentrations of IJs tested, 200 IJs showed highest mortality of aphids (cent per cent) at 48 HAT followed by 100 IJs (98.54 per cent)

Table 5: Mortality of aphids due to Isolate 1 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	38.49[e] (38.35)	45.99[e] (42.70)	59.00[c] (50.19)	70.00[c] (56.80)	79.02[b] (62.74)
50	50.00[d] (45.00)	63.51[d] (52.84)	80.02[b] (63.45)	91.58[b] (73.13)	100.00[a] (90.00)
100	68.52[c] (55.87)	82.02[c] (64.91)	98.54[a] (83.05)	100.00[a] (90.00)	100.00[a] (90.00)
200	76.01[b] (60.68)	89.56[b] (71.15)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
Dimethoate 30EC (2ml/l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD (0.05)	(1.637)	(1.802)	(3.588)	(1.544)	(0.941)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

and effect of these two treatments was statistically on par with chemical, dimethoate 30 EC. The lower concentrations (50 and 10 IJs) recorded 80.02 and 59.00 per cent mortality respectively. Effect of these two treatments was significantly different and inferior to higher inoculum levels (100 and 200 IJs).

Highest mortality of aphids (cent per cent) was recorded with 100 and 200 IJs and it was equally effective to chemical at 60 HAT. The lower concentrations of IJs (50 and 10) showed mortality percentage of 91.58 and 70.00 respectively and effect of these two treatments was inferior to 100 and 200 IJs.

Highest mortality of aphids (cent per cent) was recorded with 50, 100 and 200 IJs and it was statistically on par with chemical at 72 HAT. Lowest mortality percentage (79.02) was observed at 10 IJs level and it was inferior to all other inoculum levels.

4.3.1.3 *Tobacco Caterpillar*

Different concentrations of IJs of Isolate 1 showed statistically significant variation in the mortality of tobacco caterpillar at 24, 36, 48, 60 and 72 HAT (Table 6).

Highest mortality (cent per cent) of tobacco caterpillar was observed in chemical treatment and it was significantly superior to all other concentrations of IJs at 24 HAT. Among the different inoculum levels of IJs, 200 IJs recorded the highest mortality (22.37) followed by 100 and 50 IJs which showed 20.00 and 14.64 respectively. Effect of these three treatments was statistically on par and was inferior to chemical flubendiamide 39.35 SC. Minimum mortality (2.57 per cent) was recorded with 10 IJs and it was significantly inferior to all other concentrations of IJs.

At 36 HAT, among different concentrations of IJs, highest mortality (42.48 per cent) was observed at 200 IJ level and it was statistically on par with 100 IJs in which 37.44 percent mortality of tobacco caterpillar was observed. The mortality

Table 6: Mortality of tobacco caterpillars due to Isolate 1 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	2.57[c] (9.22)	9.44[d] (17.89)	19.48[d] (26.19)	29.75[e] (33.05)	39.89[e] (39.17)
50	14.64[b] (22.5)	24.83[c] (29.89)	39.89[c] (39.17)	50.00[d] (45)	57.52[d] (49.33)
100	20.00[b] (26.56)	37.44[b] (37.73)	57.52[b] (49.33)	65.19[c] (53.84)	73.20[c] (58.83)
200	22.37[b] (28.23)	42.48[b] (40.67)	62.56[b] (52.27)	75.17[b] (60.11)	85.36[b] (67.50)
Flubendiamide 39.35 SC (1ml/10l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD (0.05)	(8.225)	(9.348)	(5.971)	(6.745)	(8.001)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

percentage at 50 IJs level was 24.83 and it was significantly inferior to 100 and 200 IJs. Minimum mortality (9.44 per cent) was recorded with 10 IJs and it was significantly inferior to higher concentrations of IJs. Effect of chemical treatment was significantly superior to all other treatments in which cent per cent mortality was observed.

Among different inoculum levels of IJs of Isolate 1, highest mortality of tobacco caterpillar (62.56 per cent) was recorded at 200 IJ level and it was significantly superior to all other inoculum levels at 48 HAT. Treatment with 100 IJs recorded 57.52 per cent mortality and it was found statistically on par with 200 IJs. The mortality percentage of 50 IJs was recorded as 39.89 and it was inferior to 100 and 200 IJs. Minimum mortality (19.48 per cent) was recorded with 10 IJs and it was significantly inferior to higher concentrations of IJs. In chemical treatment, flubendiamide 39.35 EC, cent per cent mortality of tobacco caterpillar was observed and it was significantly superior to all other treatments.

At 60 HAT, Isolate 1 with 200 IJs recorded 75.17 per cent mortality of tobacco caterpillar and it was inferior to chemical treatment in which cent per cent mortality was observed. Lower concentration of Isolate 1 *viz.* 100, 50 and 10 IJs recorded mortality percentage of 65.19, 50.00 and 29.75 respectively. Effect of all the treatments was significantly different from each other and was inferior to 200 IJs and chemical.

Highest mortality of tobacco caterpillar was observed in chemical treatment (cent per cent) followed by different concentration levels of Isolate 1 *viz.* 200, 100, 50 and 10 IJs at 72 HAT. Treatment with 200 IJs recorded 85.36 per cent mortality and it was significantly superior to all other concentration levels. The effect of lower concentrations *viz.* 100, 50 and 10 IJs were inferior to 200 IJs giving mortality percentage of 73.20, 57.52 and 39.89 respectively

4.3.1.4 *Pseudostem Weevil*

The results presented in Table 7 revealed that there was statistically significant variation between different levels of IJs of Isolate 1 on mortality of pseudostem weevil grubs at 24, 36, 48, 60 and 72 HAT.

200 IJs of Isolate 1 recorded a per cent mortality of 5.71 and it was significantly superior to chemical (Chlorpyriphos 20EC) which showed 0.65 per cent mortality at 24 HAT. No mortality of pseudostem weevil grubs were observed at 100, 50 and 10 IJ levels and they were inferior to 200 IJs.

Considering the inoculum levels of Isolate 1, 200 IJs recorded the highest mortality with 17.24 per cent followed by 100 IJs (2.57 per cent) at 36 HAT and they were significantly different from10 and 50 IJs in which no mortality was observed. Mortality percentage of chemical (39.90) was found superior to other treatments.

Among the different concentrations of IJs of Isolate 1, 200 IJs showed highest mortality with a corrected mortality percentage of 27.38 at 48 HAT and it was significantly superior to lower concentrations of IJs *viz.* 100, 50 and 10. The corrected mortality percentage in the lower concentrations of IJs ranged from 0.00 to 17.24 and it was significantly different. Highest mortality of pseudostem weevil grubs (cent per cent) was observed in chemical treatment.

Among different concentrations of IJs of Isolate 1 tested, pseudostem weevil grubs at 200 IJ level recorded 37.44 per cent mortality followed by 100 IJs with mortality percentage of 32.43. Effect of these two treatments was statistically on par. The mortality percentage with 50 and 10 IJs were 14.65 and 5.71 respectively and they were significantly inferior to 100 and 200 IJs. Effect of chemical treatment was significantly superior to all other treatments in which cent per cent mortality was observed.

Table 7: Mortality of pseudostem weevils due to Isolate 1 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	0.00 (0.03)	0.00 (0.03)	0.00 (0.03)	5.71[d] (13.82)	21.83[c] (27.86)
50	0.00 (0.03)	0.00 (0.03)	2.57[d] (9.21)	14.65[c] (22.5)	27.38[c] (31.55)
100	0.00 (0.03)	2.57[c] (9.22)	17.24[c] (24.53)	32.43[b] (34.72)	44.97[b] (42.12)
200	5.71[a] (13.80)	17.24[b] (24.53)	27.38[b] (31.55)	37.44[b] (37.73)	50.00[b] (45.00)
Chlorpyriphos 20 EC (2ml/l)	0.65[b] (4.61)	39.90[a] (39.20)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD (0.05)	(8.863)	(8.409)	(8.069)	(7.605)	(6.577)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

Highest mortality (cent per cent) of pseudostem weevil grubs was observed in chemical treatment and it was significantly superior to all other concentrations of IJs at 72 HAT. Among the different inoculum levels of IJs of Isolate 1, 200 IJs recorded the highest mortality (50.00 per cent) followed by 100 IJs which showed 44.97 per cent mortality and effect of these two treatments was statistically on par. Inoculums levels of 50 and 10 IJs recorded a mortality percentage of 27.38 and 21.83 and they were inferior to higher concentrations of IJs (100 and 200 IJs). The effect of chemical was found superior to all concentrations of IJs.

4.3.2 Isolate 2

4.3.2.1 ***Termites***

The results presented in Table 8 showed statistically significant variation between different concentrations of IJs of Isolate 2 on mortality of termites at 24, 36, 48, 60 and 72 HAT.

The per cent mortality of termites at different concentrations of IJs was significantly different from the chemical (Chlorpyriphos 25 EC) which showed cent per cent mortality. Among the different concentrations of IJs, 200 IJs showed the highest mortality with a corrected mortality percentage of 79.04 and it was statistically superior to all other concentrations of IJs. The mortality of termites treated with 100 IJs was 68.55 percent and it was significantly superior to lower concentrations (10 IJs and 50 IJs). Treatment with 50 IJs showed 61.51 per cent mortality and it was significantly superior to 10 IJs. Minimum mortality of termites was recorded with 10 IJs and it showed a corrected mortality percentage of 49.50.

At 36 HAT, 200 IJs of Isolate 2 showed the highest mortality with a corrected mortality percentage of 99.26 and it was found statistically on par with the chemical. All other concentrations of IJs were found significantly inferior to 200 IJs. The mortality of termites treated with 100 IJs was 87.17 percent and it was significantly

Table 8: Mortality of termites due to Isolate 2 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	49.50[e] (44.71)	61.63[d] (51.73)	75.78[c] (60.52)	94.06[a] (75.89)	100.00[a] (90.00)
50	61.51[d] (51.65)	79.54[c] (63.11)	96.26[b] (78.84)	100.00[a] (90.00)	100.00[a] (90.00)
100	68.55[c] (55.89)	87.17[b] (69.01)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
200	79.04[b] (62.76)	99.26[a] (85.08)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
Chlorpyriphos 25 EC (2ml/l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD (0.05)	(2.781)	(5.744)	(4.112)	(6.805)	NS

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

superior to 50 and 10 IJs which showed a mortality percentage of 79.54 and 61.63 respectively.

Highest mortality of termites (cent per cent) was observed in 100 and 200 IJs and it was statistically on par with the chemical at 48 HAT. The corrected mortality percentage of termites observed in 50 IJs was 96.26 per cent and it was inferior to above treatments. Isolate 2 at 10 IJs level was inferior to 50 IJs and gave a mortality of 75.78 per cent.

Isolate 2 at 50 and 10 IJs level recorded 100 and 94.06 per cent mortality of termites at 60 HAT. Effect of 50 and 10 IJs was statistically on par with higher inoculum levels (100 and 200 IJs) and chemical in which cent per cent mortality was observed.

Lowest inoculum level (10 IJs) of Isolate 2 recorded cent per cent mortality of termites at 72 HAT. Effect of this treatment was statistically on par with higher inoculum levels (50, 100 and 200 IJs) and chemical check.

4.3.2.2 *Aphids*

The results presented in Table 9 revealed that there was statistically significant variation between different levels of IJs of Isolate 2 on mortality of aphids at 24, 36, 48, 60 and 72 HAT.

Among the different concentrations of IJs, 200 IJs of Isolate 2 showed the highest mortality with a corrected mortality percentage of 61.52 and it was significantly superior to lower concentrations of IJs *viz*. 100, 50 and 10. The corrected mortality percentage in the lower concentrations of IJs ranged from 21.89 to 52.00 at 24 HAT and it was significantly different from one another. Highest mortality of aphids (cent per cent) was observed in chemical treatment.

Table 9: Mortality of aphids due to Isolate 2 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	21.89[e] (27.90)	32.91[d] (35.00)	44.47[c] (41.82)	57.73[b] (49.44)	71.26[b] (57.58)
50	39.98[d] (39.22)	64.53[c] (53.44)	86.71[b] (68.62)	99.87[a] (87.97)	100.00[a] (90.00)
100	52.00[c] (46.15)	85.33[b] (67.48)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
200	61.52[b] (51.66)	99.26[a] (85.08)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
Dimethoate 30EC (2ml/l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD (0.05)	(2.967)	(5.754)	(4.277)	(5.276)	(5.521)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

200 IJs of Isolate 2 at 36 HAT showed the highest mortality with a corrected mortality percentage of 99.26 and it was found statistically on par with the chemical. All other concentrations of IJs were found significantly inferior to 200 IJs. The mortality of aphids treated with 100 IJs was 85.33 percent and it was significantly superior to 50 and 10 IJs which showed a mortality percentage of 64.53 and 32.91 respectively.

Highest mortality of aphids (cent per cent) was observed in 100 and 200 IJs and it was statistically on par with the chemical at 48 HAT. The corrected mortality percentage of aphids observed in 50 IJs was 86.71 and it was inferior to above treatments. 10 IJs of Isolate 2 was inferior to 50 IJs and gave a mortality of 44.47 per cent.

Isolate 2 at 50 IJs level recorded 99.87 per cent mortality of aphids at 60 HAT. Effect of this treatment was statistically on par with higher inoculum levels (100 and 200 IJs) and chemical. The lowest inoculum level of 10 IJs recorded 57.73 per cent mortality of aphids which was inferior to all other treatments.

50 IJs level of Isolate 2 recorded cent per cent mortality of aphids at 72 HAT. Higher inoculum levels *viz.* 100 and 200 IJs were found statistically on par with the chemical. All other treatments were found significantly superior to 10 IJs which recorded 71.26 per cent mortality of aphids.

4.3.2.3 *Tobacco Caterpillar*

Different concentrations of IJs of Isolate 2 showed statistically significant variation in the mortality of tobacco caterpillar at 24, 36, 48, 60 and 72 HAT (Table10).

Highest mortality (cent per cent) of tobacco caterpillar was observed in chemical treatment and it was significantly superior to all other concentrations of IJs at 24 HAT. Among the different inoculum levels of IJs, 200 IJs recorded the highest

Table10: Mortality of tobacco caterpillars due to Isolate 2 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	17.24[b] (24.53)	27.38[d] (31.55)	39.89[d] (39.17)	57.52[d] (49.33)	70.25[b] (56.94)
50	22.37[b] (28.23)	32.43[c] (34.72)	42.48[d] (40.67)	57.52[d] (49.33)	70.00[b] (56.79)
100	24.83[b] (29.89)	39.89[b] (39.17)	57.52[c] (49.33)	72.62[c] (58.45)	92.53[a] (74.14)
200	29.75[b] (33.05)	44.97[b] (42.12)	65.08[b] (53.78)	80.52[b] (63.81)	99.35[a] (85.39)
Flubendiamide 39.35 SC (1ml/10l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD(0.05)	(5.650)	(5.013)	(4.898)	(5.450)	(10.509)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

mortality (29.75 per cent) followed by 100 and 50 IJs which showed 24.83 and 22.37 per cent respectively. 10 IJs recorded the lowest mortality of 17.24 per cent. Effect of all the concentrations of IJs was statistically on par and was inferior to chemical flubendiamide 39.35 SC.

In tobacco caterpillar, 200 IJs recorded the highest mortality (44.97 per cent) among different concentration level of IJs at 36 HAT and it was statistically on par with 100 IJs (39.89 per cent). The mortality percentage with 50 IJs was 32.43 and it was significantly inferior to 100 and 200 IJs. Minimum mortality (27.38 per cent) was recorded with 10 IJs and it was significantly inferior to higher concentrations of IJs. Effect of chemical treatment was significantly superior to all other treatments in which cent per cent mortality was observed.

Among the different concentrations of IJs of Isolate 2, 200 IJs showed the highest mortality of tobacco caterpillar with a mortality percentage of 65.08 at 48 HAT and it was significantly superior to all other concentrations of IJs. The mortality with 100 IJs was 57.52 percent and it was significantly superior to lower concentrations (10 and 50 IJs). Mortality percentage with 50 and 10 IJs was 42.48 and 39.89 respectively and they were statistically on par. Mortality percentage of chemical treatment (cent per cent) was found to be significantly superior to all the concentrations of IJs.

200 IJs of Isolate 2 recorded 80.52 per cent mortality followed by 100 IJs (72.62 per cent) at 60 HAT and effect of these two treatments was significantly different. Lower inoculum level of 50 and 10 IJs recorded 57.52 per cent mortality of tobacco caterpillar. Effect of these two treatments was statistically on par and was inferior to higher inoculum levels of 100 and 200 IJs. Treatment with chemical was significantly superior to all other concentrations with cent per cent mortality.

Considering different concentrations of IJs of Isolate 2, the effect of 200 and 100 IJs was statistically on par with the chemical (cent per cent) with a corrected

mortality percentage of 99.35 and 92.53 respectively at 72 HAT. Effect of lower concentrations of IJs *viz*. 10 and 50 was statistically on par but inferior to higher concentrations (200 and 100 IJs) with per cent mortality of 70.25 and 70.00 respectively.

4.3.2.4 *Pseudostem Weevil*

The results presented in Table 11 revealed that there was statistically significant variation between different levels of IJs of Isolate 2 on mortality of pseudostem weevil grubs at 24, 36, 48, 60 and 72 HAT.

200 IJs of Isolate 2 recorded a per cent mortality of 5.71 and it was significantly superior to chemical (Chlorpyriphos 20 EC) which showed 0.65 per cent mortality at 24 HAT. 100, 50 and 10 IJs showed no mortality in pseudostem weevil grubs and effect of these three treatments was inferior to 200 IJs.

Among the different concentrations of IJs of Isolate 2, 200 IJs showed highest mortality with a corrected mortality percentage of 17.24 at 36 HAT and it was significantly superior to lower concentrations of IJs *viz*. 100, 50 and 10. The corrected mortality percentage in the lower concentrations of IJs ranged from 0.00 to 5.71 and it was significantly different from one another. Highest mortality of pseudostem weevil grubs (cent per cent) was observed in chemical treatment.

The per cent mortality obtained with different inoculum levels of IJs of Isolate 2 was significantly different from the chemical which showed cent per cent mortality. 200 IJs showed the highest mortality (27.38 per cent) at 48 HAT and it was significantly superior to other concentrations of IJs. The mortality of pseudostem weevil grubs with 100 and 50 IJs was 17.24 and 14.64 percent respectively and effect of these two treatments was statistically on par. Minimum mortality was observed with 10 IJs (5.71 per cent).

Table11: Mortality of pseudostem weevils due to Isolate 2 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	0.00 (0.03)	0.00 (0.03)	5.71[d] (13.83)	17.24[d] (24.53)	32.43[d] (34.71)
50	0.00 (0.03)	2.56[d] (9.22)	14.64[c] (22.5)	22.37[d] (28.23)	32.43[d] (34.71)
100	0.00 (0.03)	5.71[c] (13.83)	17.24[c] (24.53)	32.18[c] (34.56)	47.49[c] (43.56)
200	5.71[a] (13.8)	17.24[b] (24.53)	27.38[b] (31.55)	44.87[b] (42.05)	62.66[b] (52.34)
Chlorpyriphos 20 EC (2ml/l)	0.65[b] (4.61)	39.90[a] (39.2)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD (0.05)	(7.176)	(11.410)	(9.031)	(7.738)	(6.990)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

200 IJs of Isolate 2 recorded 44.87 per cent mortality of pseudostem weevil grubs followed by 100 IJs (32.18 per cent) at 60 HAT and were significantly different from each other. The effect of 50 and 10 IJs was statistically on par showing a mortality percentage of 22.37 and 17.24 respectively and these two treatments were inferior to higher concentrations of IJs (100 and 200). Chemical was found to be superior to all the concentrations of IJs.

Among the different concentrations of IJs of Isolate 2, 200 IJs showed the highest mortality of pseudostem weevil grubs with a mortality percentage of 62.66 at 72 HAT and it was significantly superior to all other concentrations of IJs. The mortality with 100 IJs was 47.49 percent and it was significantly superior to lower concentrations (10 and 50 IJs). Mortality percentage with 50 and 10 IJs was 32.43 per cent and effect of these two treatments was statistically on par. Mortality percentage of chemical treatment (cent per cent) was found to be significantly superior to all the concentrations of IJs.

4.3.3 Isolate 3

4.3.3.1 *Termites*

The results presented in Table 12 showed statistically significant variation between different concentrations of IJs of Isolate 3 on mortality of termites at 24, 36, 48, 60 and 72 HAT.

The per cent mortality of termites at different concentrations of IJs was significantly different from the chemical (Dimethoate 30 EC) which showed cent per cent mortality. Among the different concentrations of IJs of Isolate3, 200 IJs showed highest mortality with a corrected mortality percentage of 72.04 and it was significantly superior to all other concentrations of IJs. The mortality with 100 IJs was 63.01 per cent and it was significantly superior to lower concentrations (10 and 50 IJs). A mortality percentage of 41.25 was recorded with 50 IJs and it was

Table 12: Mortality of termites due to Isolate 3 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	32.48[e] (34.75)	45.99[e] (42.70)	56.51[e] (48.74)	66.03[d] (54.35)	75.09[c] (60.06)
50	41.25[d] (39.96)	52.00[d] (46.15)	63.51[d] (52.84)	73.53[c] (59.04)	81.53[b] (64.55)
100	63.01[c] (52.54)	71.06[c] (57.45)	81.10[c] (64.23)	90.15[b] (71.71)	99.26[a] (85.08)
200	72.04[b] (58.08)	83.53[b] (66.06)	92.21[b] (73.79)	100.00[a] (90.00)	100.00[a] (90.00)
Chlorpyriphos 25 EC (2ml/l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90)	100.00[a] (90.00)	100.00[a] (90.00)
CD(0.05)	(2.131)	(2.240)	(3.235)	(2.700)	(4.515)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

significantly superior to 10 IJs. Minimum mortality was recorded with 10 IJs which showed a corrected mortality percentage of 32.48.

At 36 HAT, the per cent mortality obtained with different concentrations of IJs of Isolate 3 was significantly different from the chemical which showed cent per cent mortality. Among the different concentrations of IJs, 200 IJs showed highest mortality with a corrected mortality percentage of 83.53 and it was found significantly superior to all other concentrations of IJs. The mortality of termites treated with 100 IJs was 71.06 percent and it was significantly superior to 50 and 10 IJs which showed a mortality percentage of 52.00 and 45.99 respectively.

Among the different concentrations of IJs tested, 200 IJs showed the highest mortality with a corrected mortality percentage of 92.21 at 48 HAT and it was significantly superior to lower concentrations of IJs *viz*. 100, 50 and 10. The corrected mortality percentage in the lower concentrations of IJs ranged from 56.51 to 81.10 and it was significantly different. Highest mortality of termites (cent per cent) was observed in chemical treatment.

At 60 HAT, 200 IJs of Isolate 3 showed the highest mortality (cent per cent) and it was found statistically on par with the chemical. All other concentrations of IJs were found significantly inferior to 200 IJs. The mortality of termites treated with 100 IJs was 90.15 percent and it was significantly superior to 50 and 10 IJs which showed a mortality percentage of 73.53 and 66.03 respectively.

Among the different concentrations of IJs tested, 200 IJs showed highest mortality of termites (cent per cent) at 72 HAT followed by 100 IJs (99.26 per cent) and effect of these two treatments was statistically on par with chemical. The lower concentrations (50 and 10 IJs) recorded 81.53 and 75.09 per cent mortality respectively. Effect of these two treatments was significantly different and inferior to higher inoculum levels (100 and 200 IJs).

4.3.3.2 *Aphids*

Different concentrations of IJs of Isolate 3 showed statistically significant variation in the mortality of Aphids at 24, 36, 48, 60 and 72 HAT (Table 13).

Among the different concentrations of IJs tested, 200 IJs showed the highest mortality of aphids with a corrected mortality percentage of 57.50 and it was significantly superior to lower concentrations of IJs *viz*. 100, 50 and 10. The corrected mortality percentage in the lower concentrations of IJs ranged from 20.99 to 43.99 and it was significantly different. Highest mortality of aphids (cent per cent) was observed in chemical treatment.

The per cent mortality obtained with different concentrations of IJs was significantly different from the chemical which showed cent per cent mortality at 36 HAT. Considering different concentrations of IJs of Isolate 3, 200 IJs showed the highest mortality with a corrected mortality percentage of 71.51 and it was found significantly superior to all other concentrations of IJs. The mortality of aphids treated with 100 IJs was 62.51 percent and it was significantly superior to 50 and 10 IJs which showed a mortality percentage of 41.99 and 26.99 respectively.

At 48 HAT, the per cent mortality obtained with different concentrations of IJs of Isolate 3 was significantly different from the chemical (cent per cent). Among the different concentrations of IJs, highest mortality of aphids was recorded by 200 IJs with a mortality percentage of 91.87 and all other concentrations of IJs were found inferior to it. The mortality of aphids treated with 100 IJs was 76.05 percent and it was significantly superior to 50 (52.00 per cent) and 10 IJs (37.49 per cent).

200 IJs of Isolate 3 showed the highest mortality (cent per cent) at 60 HAT and it was found statistically on par with the chemical. All other concentrations of IJs were found significantly inferior to 200 IJs. 100 IJs resulted a mortality percentage of

Table 13: Mortality of aphids due to Isolate 3 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	20.99 e (27.27)	26.99 e (31.30)	37.49 e (37.75)	52.50 d (46.43)	67.63 c (55.32)
50	29.99 d (33.20)	41.99 d (40.39)	52.00 d (46.15)	67.04 c (54.97)	82.58 b (65.33)
100	43.99 c (41.55)	62.51 c (52.25)	76.05 c (60.69)	88.15 b (69.86)	99.27 a (85.08)
200	57.50 b (49.32)	71.51 b (57.74)	91.87 b (73.44)	100.00 a (90.00)	100.00 a (90.00)
Dimethoate 30EC (2ml/l)	100.00 a (90.00)	100.00 a (90.00)	100.00 a (90.00)	100.00 a (90.00)	100.00 a (90.00)
CD (0.05)	(1.330)	(1.795)	(3.540)	(2.734)	(5.022)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

88.15 and it was significantly superior to 50 and 10 IJs which showed a mortality percentage of 67.04 and 52.50 respectively.

Among the different concentrations of IJs of Isolate 3 tested, 200 IJs showed highest mortality of aphids (cent per cent) at 72 HAT followed by 100 IJs (99.27 per cent) and effect of these two treatments was statistically on par with chemical. The lower concentrations (50 and 10 IJs) recorded 82.58 and 67.63 per cent mortality respectively. Effect of these two treatments was significantly different and was inferior to higher inoculum levels (100 and 200 IJs).

4.3.3.3 *Tobacco Caterpillar*

The results presented in Table 14 showed statistically significant variation between different concentrations of IJs of Isolate 3 on mortality of tobacco caterpillar at 24, 36, 48, 60 and 72 HAT.

Highest mortality (cent per cent) of tobacco caterpillar was observed in chemical treatment and it was significantly superior to all other concentrations of IJs at 24 HAT. Among the different inoculum levels of IJs of Isolate 3, 200 IJs recorded the highest mortality (27.13 per cent) followed by 100 IJs which showed 24.83 per cent and effect of these two treatments was statistically on par. Both 50 and 10 IJs recorded a mortality percentage of 12.23 and these two treatments were inferior to higher concentartions of IJs (100 and 200). The effect of chemical flubendiamide 39.35 SC was found superior to all concentrations of IJs.

Among the different concentrations of IJs of Isolate 3, 200 IJs showed highest mortality of tobacco caterpillar with a mortality percentage of 42.48 at 36 HAT and it was significantly superior to all other concentrations of IJs. The mortality with 100 IJs was 32.43 percent and it was significantly superior to lower concentrations (10and 50 IJs). Mortality percentage at 50 and 10 IJs was 20.00 and 22.37 per cent respectively and they were statistically on par to each other. Mortality percentage of

Table14: Mortality of tobacco caterpillars due to Isolate 3 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	12.23[c] (20.47)	22.37[d] (28.23)	29.75[d] (33.05)	37.33[e] (37.66)	50.00[e] (45.00)
50	12.23[c] (20.47)	20.00[d] (26.57)	32.43[d] (34.72)	47.48[d] (43.56)	57.52[d] (49.33)
100	24.83[b] (29.89)	32.43[c] (34.72)	42.48[c] (40.67)	60.00[c] (50.77)	80.00[c] (63.43)
200	27.13[b] (31.39)	42.48[b] (40.67)	55.02[b] (47.88)	65.08[b] (53.78)	85.35[b] (67.50)
Flubendiamide 39.35 SC (1ml/10l)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD(0.05)	(6.264)	(3.625)	(5.053)	(4.921)	(4.928)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

chemical treatment (cent per cent) was found to be significantly superior to all the concentrations of IJs.

200 IJs of Isolate 3 recorded 55.02 per cent mortality followed by 100 IJs (42.48 per cent) at 48 HAT and were significantly different from each other. The effect of 50 and 10 IJs was statistically on par which recorded a mortality percentage of 32.43 and 29.75 respectively and these two treatments were inferior to higher concentrations of IJs (100 and 200). Chemical was found to be superior to all the concentrations of IJs.

At 60 HAT, Isolate 3 with 200 IJs recorded 65.08 per cent mortality of tobacco caterpillar and it was inferior to chemical treatment in which cent per cent mortality was observed. Lower concentration of Isolate 1 *viz.* 100, 50 and 10 IJs recorded mortality percentage of 60.00, 47.48 and 37.33 respectively. Effect of all the treatments was significantly different from each other and was inferior to 200 IJs and chemical.

Highest mortality of tobacco caterpillar was observed in chemical treatment (cent per cent) followed by different concentrations of Isolate 3 *viz.* 200, 100, 50 and 10 IJs at 72 HAT. Treatment with 200 IJs recorded 85.35 per cent mortality and it was significantly superior to all other concentration levels. The effect of lower concentrations *viz.* 100, 50 and 10 IJs was inferior to 200 IJs giving mortality percentage of 80.00, 57.52 and 50.00 respectively.

4.3.3.4 *Pseudostem Weevil*

Different concentrations of IJs of Isolate 3 showed statistically significant variation in the mortality of pseudostem weevil grubs at 24, 36, 48, 60 and 72 HAT (Table 15).

No mortality of pseudostem weevil grubs was observed in different concentrations of Isolate 3 (10, 50, 100 and 200 IJs) at 24 HAT. Chemical treatment

Table15: Mortality of pseudostem weevils due to Isolate 3 of EPN

Dose (IJ)	Mortality* (%) Hours after treatment (HAT)				
	24	36	48	60	72
10	0.00 (0.03)	0.00 (0.03)	0.00 (0.03)	5.71[c] (13.83)	20.00[d] (26.57)
50	0.00 (0.03)	0.00 (0.03)	0.00 (0.03)	5.71[c] (13.87)	21.83[d] (27.85)
100	0.00 (0.03)	0.00 (0.03)	5.71[b] (13.87)	17.24[c] (24.53)	29.75[c] (33.05)
200	0.00 (0.03)	0.00 (0.03)	10.00[b] (18.43)	27.38[b] (31.55)	37.44[b] (37.73)
Chlorpyriphos 20 EC (2ml/l)	0.65 (4.61)	39.90[a] (39.2)	100.00[a] (90.00)	100.00[a] (90.00)	100.00[a] (90.00)
CD(0.05)	NS	(3.273)	(6.267)	(9.555)	(6.272)

Figures in parenthesis are arc sine transformed values

*Mortality corrected using Abbott's formula

with Chlorpyriphos 20 EC recorded 0.65 per cent mortality of pseudostem weevil grubs.

At 36 HAT, no mortality of pseudostem weevil grubs was observed in different concentrtaions of IJs (10, 50, 100 and 200 IJs) while in chemical treatment 39.90 per cent mortality was observed.

All the treatments were significantly different from chemical which showed a mortality of cent per cent. Among the different concentrations of IJs of Isolate 3 tested, 200 IJs recorded the highest mortality (10.00 per cent) followed by 100 IJs (5.71 per cent) at 48 HAT and effect of these treatments was statistically on par to one another. 10 and 50 IJs were inferior to higher concentrations of IJs (100 and 200 IJs) and no mortality of pseudostem weevil grubs was observed in these treatments.

At 60 HAT, effect of all inoculum levels of IJs of Isolate 3 was significantly different from the chemical which recorded cent per cent mortality of pseudostem weevil grubs. The highest mortality was recorded with 200 IJs (27.38 per cent) and it was significantly superior to all other lower concentrations. The per cent mortality of 100, 50 and 10 IJs was recorded as 17.24, 5.71 and 5.71 per cent respectively and effect of these treatments was statistically on par.

Among the different concentrations of IJs of Isolate 3, 200 IJs recorded the highest mortality (37.44 per cent) followed by 100 IJ (29.75 per cent) at 72HAT. 100 IJs recorded a per cent mortality of 29.75 and it was significantly different from the mortality percentage of 200 IJs. The effect of 50 and 10 IJs was found to be statistically on par with mortality percentage of 21.83 and 20.00 respectively. Highest mortality (cent percent) was recorded in chemical treatment and it was superior to all inoculum levels.

4.4 MORPHOLOGICAL CHARACTERS OF EPN ISOLATES

Morphological and morphometric studies of these native isolates were undertaken and their salient features were analyzed for identifying the similarities and differences between them. The characteristic features along with morphometric measurements of each isolate are presented in the Table 16-22.

4.4.1 Isolate 1 (Vellayani strain)

4.4.1.1 *Infective Juveniles (IJs)*

Infective juveniles had an elongate and thin body, excretory pore was indistinct. They had a closed mouth opening. The 2nd stage cuticles were retained in their body. Oesophagus had a cylindrical procorpus and slightly swollen metacorpus. Tail was elongate and gradually tapering (Plate 17A).

4.4.1.2 *Females*

Females were C shaped upon fixation and was characterized by a smooth cuticle (Plate 17B). Lips were united and slightly rounded. Stoma was short. Muscular oesophagus (165.60±6.74µm) with procorpus slightly expanded in the anterior portion just behind the stoma and had a slightly enlarged metacorpus (Plate 18A). Isthmic region was short and narrowing with a nerve ring anterior to the basal bulb. Basal bulb was round and had a small valve. Excretory pore was present anterior to the nerve ring (154.00±5.57µm) (Plate 18B). Gonads were didelphic, amphidelphic and reflexed. Vulva was seen as a transverse slit situated on a protruberance and was submedially located (Plate 18C). Tail (54.10±5.26µm) (Plate 18D) was conical without a mucron at the terminus (Table 16).

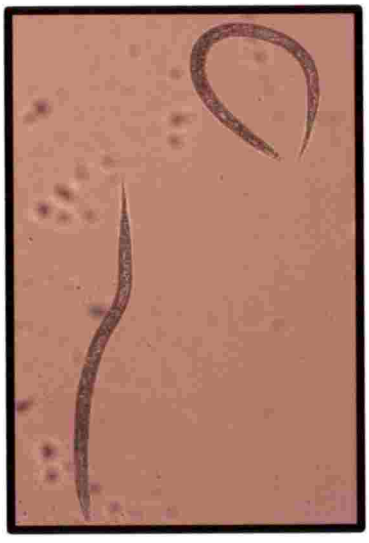
4.4.1.3 *Males*

The male body was J-shaped, slender and ventrally curved when killed in a hot fixative (859.10±42.43) (Plate 17C). Lip region was continuous with a short stoma. Muscular oesophagus with cylindrical procorpus (144.40±4.14µm). Isthmus

Table 16: Morphometric characters of females of isolate 1 (Mean±SD), n=15

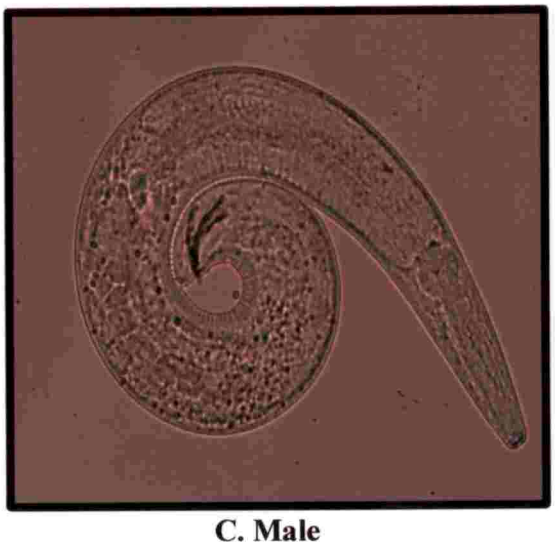
Characters	Measurements (µm)
Body length	2133.88±87.11 (1866-2466)
Body diameter	114.80±4.21 (104-136)
EP	154.00±5.57 (148-159)
ES	165.60±6.74 (158-175)
Vulval %	48.40±2.17 (45-51)
ABD	23.90±2.85 (20-27)
Tail Length	54.10±5.26 (48-61)
a=L/W	17.40±1.06 (15.82-18.51)
b=L/ES	7.13±0.60 (6.23-8.15)
c=L/T	7.10±0.56 (6.03-7.63)
D%= EP/ES*100	95.90±3.42 (91-97)
E%=EP/T*100	278.14±9.12 (246-288)

Figures in parenthesis show Range



A. Infective Juveniles

B. Female



C. Male

Plate 17. Isolate 1 (*Steinernema* sp.)

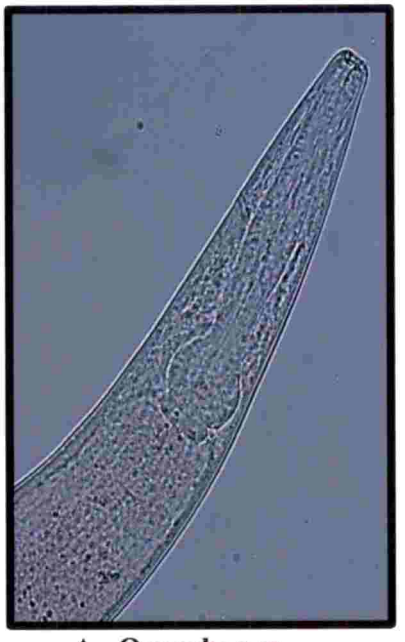
Plate 18

A- Anterior region- Oesophagous (40X)

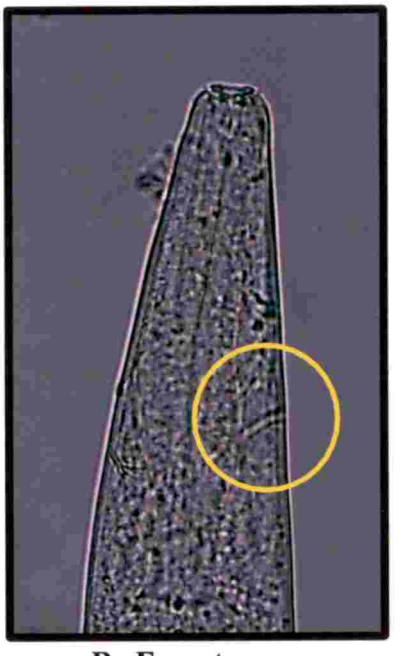
B- Anterior region showing excretory pore (40X)

C- Vulval region showing double flapped epiptygma (100X)

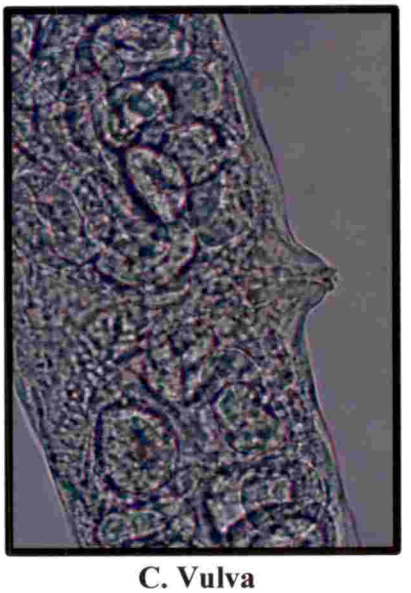
D- Tail with slight post anal swelling (100X)



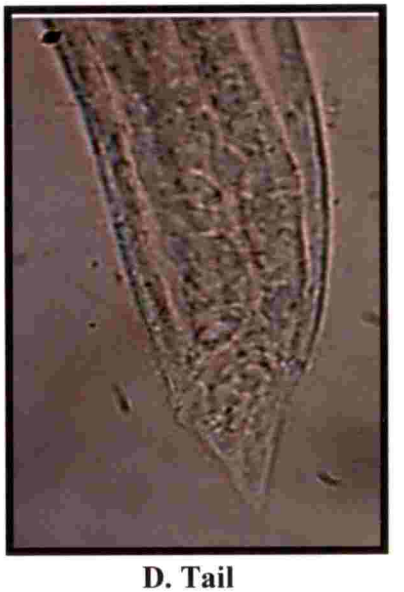
A. Oesophagus



B. Excretory pore



C. Vulva



D. Tail

Plate 18. Morphological characters of Isolate 1 (*Steinernema* sp.) females

was narrow with nerve ring encircling the region. The basal bulb was round. Excretory pore was present just above the nerve ring. Spicule (44.10±4.46µm) was paired, ventrally curved and slightly swollen in the middle with a boat shaped gubernaculum (23.70±2.98µm) (Plate 19A). Bursa absent at the posterior end. Tail (35.80±5.20µm) (Plate 19B) was conoid with a mucron at the tail terminus (Table 17).

Based on the morphological and morphometric characters, Isolate 1 was identified as *Steinernema* sp.

4.4.2 Isolate 2 (Mylom strain)

4.4.2.1 *Infective Juveniles*

IJs had a narrow and elongated body when killed by heat. The 2nd stage cuticles were not retained in their body. The tail was long and pointed and covered with a sheath (Plate 20A).

4.4.2.2 *Hermaphrodites*

Hermaphrodites (1374.26±63.00µm) had a C-shaped body when killed in hot fixative (Plate 20B). The labial region had six well-developed labium (two dorsal sectors, right ventral and sub ventral sectors, left ventral and sub ventral sectors). The stoma was tubular, and the cheilorhabdions were well cuticularized (Plate 21A). They had a pharynx with a cylindrical corpus throughout (198.50±8.44µm). The isthmus was long and distinguishable. The valve of the basal bulb was prominent. Gonads were didelphic, amphidelphic, and reflexed. The vulva was a transverse slit situated on a protruding area usually near midbody. The tail had a conoid (98.70±9.03µm)or post-anal swelling with a pointed terminus (Plate 21B). The reproductive system was amphidelphic and reflexed (Table 18).

Table 17: Morphometric characters of males of isolate 1 (Mean±SD), n=15

Characters	Measurements (µm)
Body length	859.10±42.43 (801-901)
Body diameter	46.70±4.00 (40-52)
EP	-
ES	144.40±4.14 (140-151)
Tail Length	35.80±5.20 (28-41)
Spicule length	44.10±4.46 (40-51)
Gubernaulum	23.70±2.98 (20-29)
SW=SL/ABD*100	186.99±29.45 (168.00-238.09)
GS=GL/SL*100	54.28±9.43 (42.00-52.38)
a=L/W	18.52±1.77 (15.40-20.60)
b=L/ES	5.95±0.33 (5.56-6.43)
c=L/T	24.36±2.89 (20.61-28.60)
D%= EP/ES*100	-
E%=EP/T*100	-

Figures in parenthesis show Range

Table 18: Morphometric characters of hermaphrodites of isolate (Mean±SD), n=15

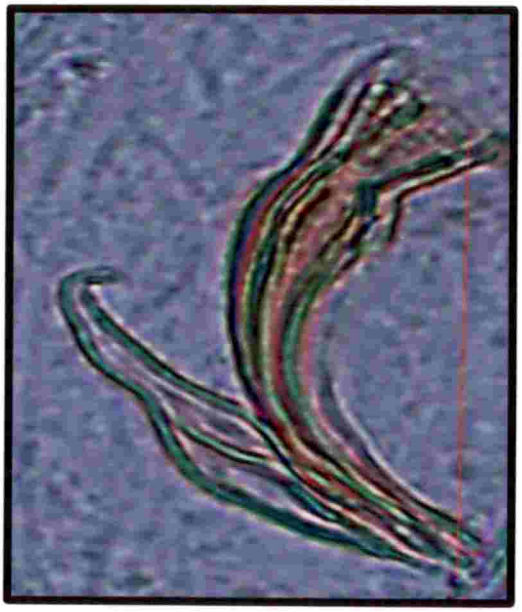
Characters	Measurements (µm)
Body length	1374.26±63.00 (1236-1547)
Body diameter	86.13±6.55 (79-98)
EP	132.33±6.51 (126-148)
ES	198.50±8.44 (162-208)
Vulval %	46.17±2.32 (43-49)
ABD	40.30±4.08 (34-48)
Tail Length	98.70±9.03 (86-106)
a=L/W	12.51±0.69 (11.48-13.45)
b=L/ES	8.63±0.66 (7.37-9.63)
c=L/T	11.86±1.85 (9.59-12.77)
D%= EP/ES*100	64.81±5.26 (58.74-68.10)
E%=EP/T*100	198.25±6.69 (182.25-208.50)

Figures in parenthesis show Range

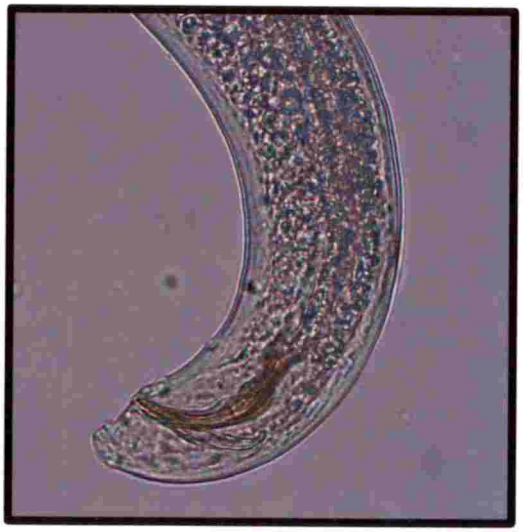
Plate 19

A- Paired and ventrally curved spicules and boat shaped gubernaculum (100X)

B- Posterior region showing tail (40X)



A. Spicules and Gubernaculum



B. Tail

Plate 19. Morphological characters of Isolate 1 (*Steinernema* sp.) males

4.4.2.3 *Females*

Females had a long and elongated body (1308.70±95.22µm) (Plate 20C). The labial region had six labial papillae, lacking visible cephalic papillae. The stoma was tubular, and the cheilorhabdions were not cuticularized. The metacarpus was slightly swollen and the oesophageal collar (procorpus) was very long. The isthmus was distinct with a pyriform basal bulb and a prominent butterfly valve (215.78±10.60µm). Presence of lobed oesophageal glands which were protruding into the intestine. The excretory pore was located anterior to the basal bulb (146.50±15.18µm). They had a slightly protruding vulval lip which was seen near to the anal slit (Plate 22A). The tail (166.50±12.11µm) (Plate 22B) was filiform which was longer than the anal body diameter (ABD) (Table 19).

4.4.2.4 *Males*

Males had a J-shaped body (696.60±73.89µm) when killed in hot fixative (Plate 20D). The labial region had six labial papillae. The stoma was tubular/ funnel shaped, and the cheilorhabdions were not cuticularized (Plate 23A). The metarhabdions showed no hemispherical swellings. They had a cylindrical procorpus and slightly swollen metacorpus. The isthmus was distinct with a pyriform basal bulb and a prominent valve (138.00±5.33µm). Presence of lobed oesophageal glands which were protruding into the intestine (Plate 23B). The excretory pore was located anterior to the basal bulb (109.00±6.35µm). The tail was short, pointed and conoid (28.60±5.10µm) (Plate 23C).The bursa was peloderan extending on both sides and surrounds the male cloaca and the bursal rays were well developed with eight genital papillae. The spicules (39.63±3.59µm) were paired, separate, often asymmetrical, and slightly curved ventrally. Gubernaculum (28.00±3.86µm) slightly curved ventrally and had a length more than half the spicule length (Plate 23D). The testis was monorchic and reflexed. The head of the spicule was round (round manubrium) (Table 20).

Table 19: Morphometric characters of females of isolate 2 (Mean±SD), n=15

Characters	Measurements (µm)
Body length	1308.70±95.22 (1280-1634)
Body diameter	54.90±4.84 (48-64)
EP	146.50±15.18 (138-176)
ES	215.78±10.60 (206-234)
Vulval %	66.50±5.26 (62-74)
ABD	31.20±6.03 (25-45)
Tail Length	166.50±12.11 (146-180)
a=L/W	25.91±3.65 (21.16-34.04)
b=L/ES	6.73±0.74 (5.83-8.71)
c=L/T	8.43±2.89 (7.33-9.01)
D%= EP/ES*100	64.61±7.96 (60.55-76.24)
E%=EP/T*100	70.48±6.97 (61.03-80.50)

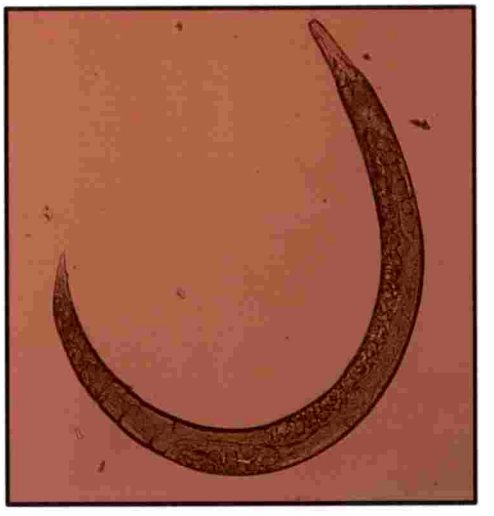
Figures in parenthesis show Range

Table 20: Morphometric characters of males of isolate 2 (Mean±SD), n=15

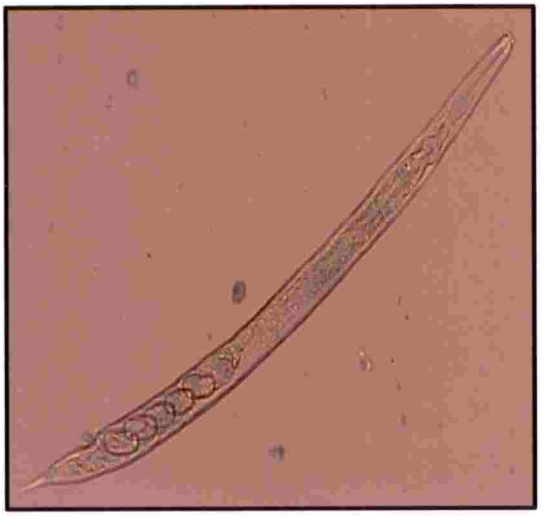
Characters	Measurements (µm)
Body length	696.60±73.89 (644-860)
Body diameter	54.30±6.72 (48-74)
EP	109.00±6.35 (98-135)
ES	138.00±5.33 (134-156)
Tail Length	28.60±5.10 (21-37)
Spicule length	39.63±3.59 (34-46)
Gubernaulum	28.00±3.86 (24-37)
SW=SL/ABD*100	186.99±29.45 (168.00-238.09)
GS=GL/SL*100	146.11±29.20 (110.81-190.48)
a=L/W	54.51±8.25 (42.86-66.67)
b=L/ES	10.80±1.93 (9.6-15.4)
c=L/T	3.90±0.50 (3.4-5.7)
D%= EP/ES*100	19.16±3.07 (18.8-26.6)
E%=EP/T*100	85.29±0.92 (84.44-86.36)

Figures in parenthesis show Range

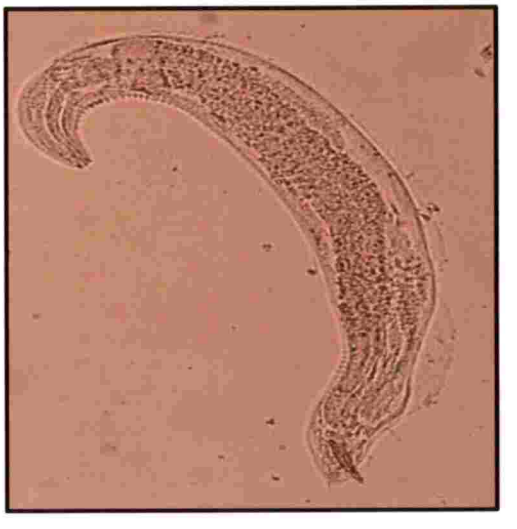
A. Infective Juvenile



B. Hermaphrodite



C. Female



D. Male

Plate 20. Isolate 2 (*Metarhabditis rainai*)

Plate 21

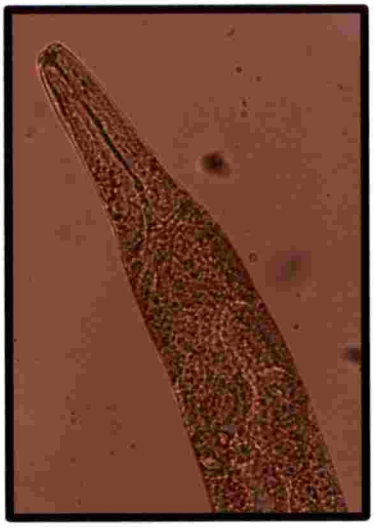
A- Anterior region showing tubular stoma (40X)

B- Posterior region showing tail with post anal swelling (40X)

Plate 22

A- Vulva seen near to the anal slit (100X)

B- Posterior region showing filiform tail (40X)

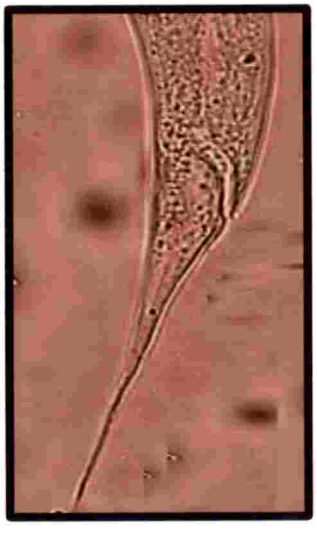


A. Stoma

B. Tail

Plate 21. Morphological characters of Isolate 2 (*Metarhabditis rainai*) hermaphrodite

A. Vulva



B. Tail

Plate 22. Morphological characters of Isolate 2 (*Metarhabditis rainai*) female

Plate 23

A- Anterior region showing tubular stoma (40X)

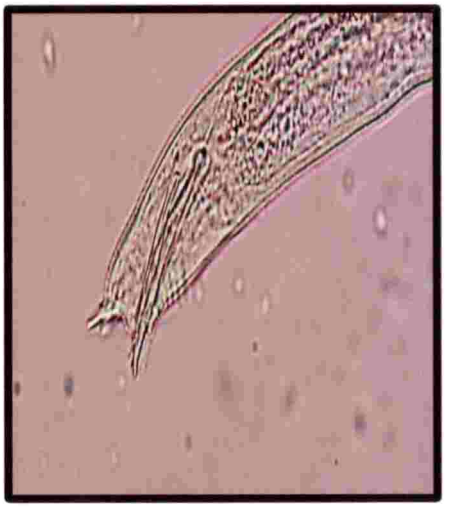
B- Lobed oesophageal gland overlapping the intestine (100X)

C- Posterior region showing short, pointed tail (40X)

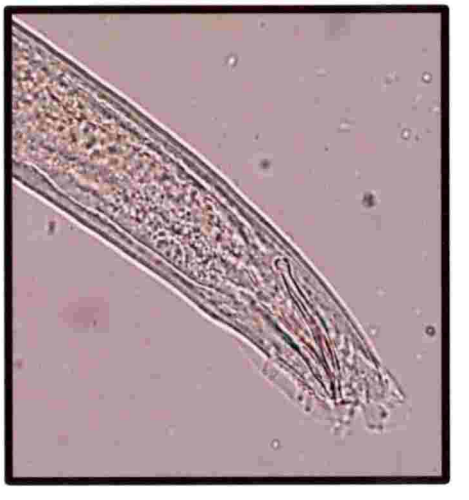
D- Posterior region showing peloderan bursa with 8 genital papillae (40X)

A. Stoma

B. Oesophageal glands



C. Tail



D. Spicule, Gubernaculum and Bursa

Plate 23. Morphological characters of Isolate 2 (*Metarhabditis rainai*) male

Based on the morphological and morphometric characters and molecular analysis, Isolate 2 was identified as *Metarhabditis rainai*. This is the first report of *M. rainai* in India.

4.4.3 Isolate 3 (Kainidi strain)

The isolate was obtained from Kainidi, Alappuzha. The characteristics of this native isolate were similar to the males and females of Isolate 2 obtained from Mylom, Kottarakara. Most of morphological characters were similar with some differing characters.

4.4.3.1 *Infective Juveniles*

IJs have an elongated body when heat killed. Their cuticles are slightly annulated. The tail is long and pointed and covered with a sheath (Plate 24A).

4.4.3.2 *Females*

Females had a swollen body (1337.14±47.18µm) when killed by heat (Plate 24B). The stoma was tubular, and the cheilorhabdions were well cuticularized. The isthmus was long and distinguishable, and the nerve ring surround the posterior part of isthmus (Plate 25A). The butterfly valve of the basal bulb was prominent (208.14±5.64µm). The oesophageal glands were not distinct. The excretory pore was located anterior to the basal bulb (139.25±5.50µm). Gonads were didelphic, amphidelphic, and reflexed. The vulva was a transverse slit situated on a protruding area near to the anal region. It has two symmetrical vulval lips and a short vagina. The tail (Plate 25B) is filiform (112.5±4.64µm) without a post-anal swelling (Table 21).

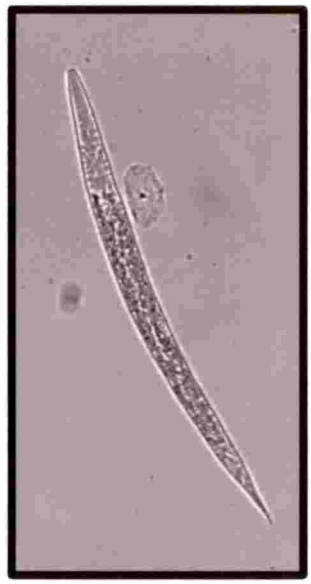
4.4.3.3 *Males*

Males had a J-shaped body (804.67±46.34µm) upon fixation (Plate 24C). Six separate well-developed lips were present (Plate 26A). They had a typical Rhabditoid oesophagus with long procopus and swollen metacorpus. Isthmus was distinct with a globose basal bulb and a prominent valve (84.71±5.35µm). The nerve ring surrounding the isthmus was located posterior to it. Cardia was clearly visible (Plate

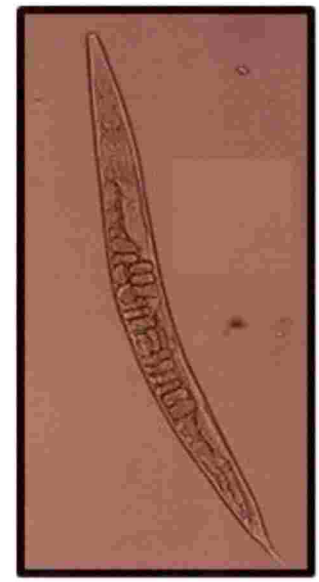
Table 21: Morphometric characters of females of isolate 3 (Mean±SD), n=15

Characters	Measurements (µm)
Body length	1337.14±47.18 (1256-1394)
Body diameter	53±6.34 (43-59)
EP	139.25±5.50 (122-154)
ES	208.14±5.64 (202-216)
Vulval %	67.38±5.38 (59-75)
ABD	29.43±4.43 (24-35)
Tail Length	112.5±4.64 (106-121)
a=L/W	12.60±2.08 (10.95-14.07)
b=L/ES	7.35±0.48 (6.54-8.05)
c=L/T	10.73±2.21 (9.08-12.85)
D%= EP/ES*100	67.02±1.94 (58.93-76.12)
E%=EP/T*100	248.18±6.27 (228.12-264.72)

Figures in parenthesis show Range

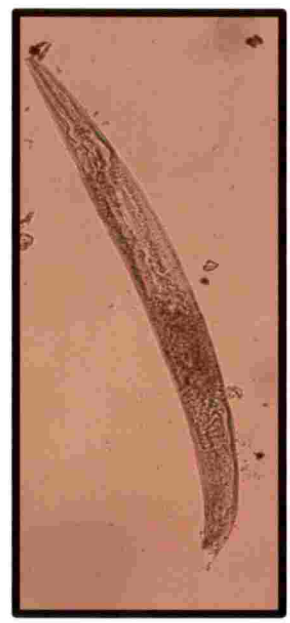


A. Infective Juvenile



B. Female

B



C. Male

Plate 24. Isolate 3 (*Rhabditis* sp.)

Plate 25

A- Anterior region showing oesophagous (40X)

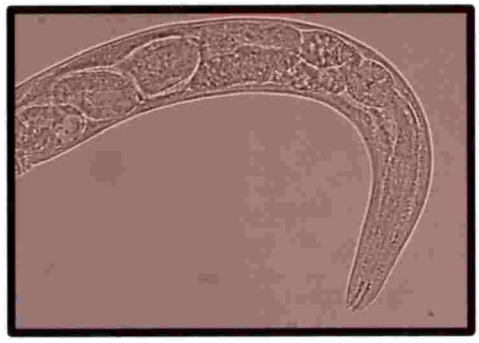
B- Posterior region showing filiform tail and transverse vulval slit (40X)

Plate 26

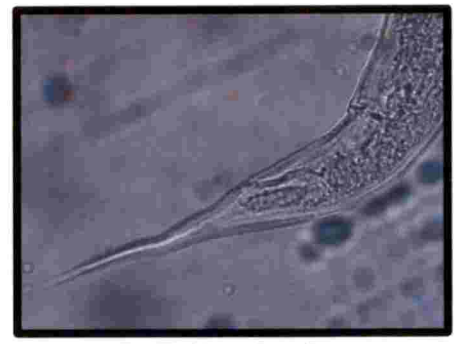
A- Anterior region showing tubular stoma (100X)

B- Cardia below the basal bulb (100X)

C- Tail region showing leptoderan bursa with bursal rays (100X)

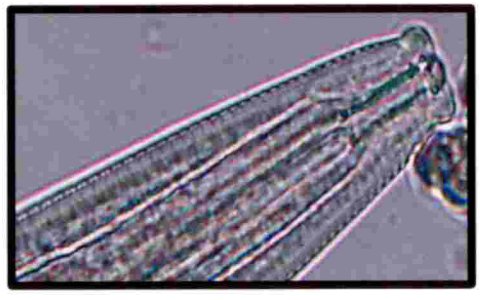


A. Oesophagous

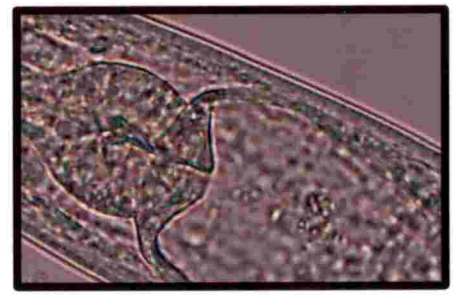


B. Tail and vulva

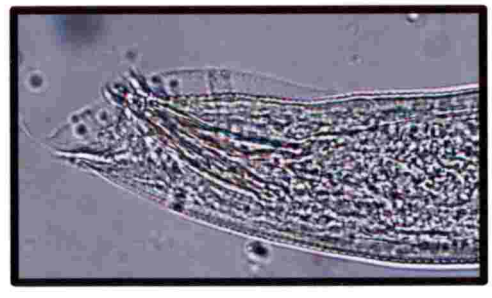
Plate 25. Morphological features of Isolate 3 (*Rhabditis* sp.) females



A. Stoma



B. Cardia



C. Spicule, Gubernaculum and Bursa

Plate 26. Morphological features of Isolate 3 (*Rhabditis* sp.) males

26B). Bursa was leptoderan with well developed bursal rays. The spicules (33.83±4.36µm) were paired, separate, often asymmetrical, and slightly curved ventrally. The gubernaculum (28.88±4.26µm) was boat-shaped and ventrally curved. The head of the spicule tip was not round (Plate 26C). They had a small and pointed tail (29.71±4.86µm) (Table 22).

Based on the morphological and morphometric characters, Isolate 3 was identified as *Rhabditis* sp.

4.5 MOLECULAR CHARACTERIZATION

Based on the pathogenicity studies, Isolate 2 was identified as the most potent EPN strain which caused highest mortality in termites, aphids, tobacco caterpillar and pseudostem weevil. Hence molecular characterization of Isolate 2 was done by amplifying the internal transcribed region of the isolate using TTGATTACGTCCCTGCCCTTT as forward primer and TTTCACTCGCCGTTACTAAGG as reverse primer (Plate 27). Blast analysis result revealed that Isolate 2 was *Metarhabditis rainai* (Table 23 and 24).

Table 22: Morphometric characters of males of isolate 3 (Mean±SD), n=15

Characters	Measurements (µm)
Body length	804.67±46.34 (741-862)
Body diameter	36.43±5.09 (29-39)
EP	71.67±4.72 (68-77)
ES	84.71±5.35 (78-94)
Tail Length	29.71±4.86 (24-38)
Spicule length	33.83±4.36 (29-39)
Gubernaulum	28.88±4.26 (22-37)
SW=SL/ABD*100	119.03±24.10 (100-150)
GS=GL/SL*100	55.29±10.98 (41.03-67.74)
a=L/W	22.80±2.31 (18.76-26.38)
b=L/ES	9.72±0.70 (9.00-10.96)
c=L/T	28.66±4.26 (21.29-35.91)
D%= EP/ES*100	85.91±3.92 (81.91-89.74)
E%=EP/T*100	272.25±32.37 (248.38-309.09)

Figures in parenthesis show Range

Table 23. Blast search details of the 18S rRNA sequence of superior isolate and other similar species in NCBI data base

Isolates	Description	Max score	Total score	Query cover	E value	Identity
1	Isolate 2 (*Metarhabditis rainai* PT-R14B)	835	835	100%	0.0	99.78%
2	*Metarhabditis rainai* PS1191	835	835	100%	0.0	99.78%
3	*Pellioditis marina* strain SB178	706	706	100%	0.0	94.73
4	*Rhabditella axei* isolate RhabAxe	662	662	99%	0.0	93.17

Table 24. Analysis of nucleotide sequence of Isolate 2 (*Metarhabditis rainai*)

	Sequence
Isolate 2	AAGACCAGCTGATTCTTCCAGTGTAGCGCGCGTGCAGCCCCGA ACATCTAAGGGCATCACAGACCTGTTATCGCTCAATCTCGTGCT GTTTTAACACAGCTTATTCCTCTAAGAAGTTAACTGGACTTAATT ATCCAGAAACTATTTAGTAGGTTAGAGTCTCGCTCGTTATCGGA ATAAACCAGACAAATCACTCCACCAACTAAGAACGGCCATGCA CCACCATCCACCGAATCAAGAAAGAGCTTTCAATCTGTCAATCC TCACGGTGTCCGGGCCGGGTGAGTTTTCCCGTGTTGAGTCAAA TTAAGCCGCAGGCTCCACGCCTGGTGGTGCCCTTCCGTCAATTT CTTTAAGTTTCAGCTTTGCAACCATACTACCCCAGGAACCGAAA GACTTTCGTTTCCGGGAAGCTCCCCTCCGAGACATAGATATCCC GTTGGATCGCT GATGG

Plate 27. Gel profile of the PCR product of 18s rRNA

Discussion

5. DISCUSSION

Use of biocontrol agents is an ideal and effective practice for controlling the insect pests without deteriorating the environment. EPN is one among the biocontrol agents which has proved successful and can be an alternative in the management of insect pests. Due to their potential in quick killing of the host insects, their effects can be compared with pesticides. Therefore it is rational to evaluate the ability of locally adapted isolates in controlling pests prevalent in an area. Potent EPN isolates can be mass produced and applied using conventional spray equipments. The present study was conducted to isolate native EPN strains distributed in the rhizosphere of different crops and to evaluate their pathogenicity to termites, aphids, tobacco caterpillar and pseudostem weevil. The result obtained is discussed below.

5.1. ISOLATION AND IDENTIFICATION OF INDIGENOUS EPNs

A random survey was conducted in different districts of Kerala *viz.*, Thiruvananthapuram, Kollam, Pathanamthitta and Alappuzha and a total of forty soil samples were collected from the rhizosphere of vegetables, banana and coconut. The present study revealed the natural occurrence of EPN in Kerala. In this study three isolates of EPN were obtained from Vellayani, Mylom and Kainidi areas of Kerala with 10 per cent frequency of occurrence. From the collected samples three native isolates were obtained. Sosamma and Rasmi (2002) collected 430 soil samples and found that 129 soil samples were positive for EPN with 30 per cent frequency of occurrence. *Heterorhabditis indica* occurred in 128 samples and *Steinernema* sp. in only one sample with frequency of occurrence of 90 and 0.8 percent respectively. A recent survey conducted by Anes *et al.* (2018) reported that out of 141 soil samples collected from Kollam, Pathanamthitta and Alappuzha, 13.5% were found positive for the presence of EPN. They also reported that soil samples collected from plots of ICAR-CPCRI, Kayamkulam recorded 33.3% of total number of EPN isolates. Remya (2007) isolated 10 EPN isolates from banana rhizosphere in Vellayani. So results of

the study revealed that the native isolates of EPNs prevalent in Kerala can be utilized for the management of insect pests without introducing new strains.

The isolates obtained from soil were identified based on morphological characters, taxonomic keys and morphometric measurements. Isolate 1 obtained from COA, Vellayani was identified as *Steinernema* sp. The IJs had an elongate and thin body which retained the second stage cuticle. They had closed mouth opening. Females were C shaped and males were J shaped. The anterior region of both males and females had rounded lip with short stoma. The esophagus of both males and females was muscular with cylindrical procorpus, slightly enlarged metacorpus, short and narrow isthmus surrounded by a nerve ring and rounded basal bulb. The excretory pore was seen anterior to the nerve ring in both males and females. Vulva was seen as a transverse slit situated on a protuberance and was sub medially located. The males had symmetrical, slightly curved and paired spicules. Beneath the spicule, a boat shaped gubernaculum was present. The females had a short and conoid tail with a pointed tip. The male tail was conoid with a mucron and without bursa. The above mentioned characters are shown in Plates 17, 18 and 19.

The length of female body of *Steinernema* sp. (Vellayani strain) was longer than *S. abbasi* (2133.88µm vs 541µm). The shape of spicule and gubernaculum was different from that of *S. abbasi* (Elawad *et al.*, 1997). The spicule length was similar to that of *S. abbasi* (44.10 vs 45 µm) but shorter than *S. akhursti* (90µm), *S. feltiae* (70µm), *S. masoodi* (53µm) and *S. sangi* (63µm). Gubernaculum was found shorter (23.70 vs 38.8µm) than *S. abbasi* but similarity was seen only with *S. kraussei* (29µm). The males of *Steinernema* sp. (Isolate 1) had shorter tail as compared to *S. abbasi* tail (35.80 vs 40-45µm). *Steinernema* sp. (Isolate 1) lacks mucron in first generation females similar to *S. abbasi*. *Steinernema* sp. (Isolate 1) had a continuous lip region similar to *S. masoodi*. *Steinernema* sp. (Isolate 1) had a similar E% value (278.14 vs 275µm) of *S. masoodi* (Table 16) (Ali *et al.*, 2005).

Isolate 2 obtained from Mylom was identified as *Metarhabditis rainai* which was characterised by a hermaphroditic condition in the first generation followed by males and female in the next generations. The stoma was tubular/ funnel shaped in hermaphrodites, males and females. Hermaphrodite had a cylindrical corpus throughout whereas the males and females had a typical "Rhabditoid oesophagous" with a cylindrical procorpus, undifferentiated metacorpus, distinct isthmus and pyriform shaped basal bulb with well-developed butterfly valve. Hermaphrodites had a conoid tail with post-anal swelling and pointed terminus. Females had a filiform tail and males had a short and pointed tail. Vulva was a tranverse slit with slightly protruding vulval lips and was seen near to the anal slit. The male tail had a peloderan bursa with eight genital papillae. The spicules were paired and asymmetrical and slightly curved ventrally. Gubernaculum was slightly curved ventrally and had a length more than half the spicule length. The above mentioned characters are shown in Plates 20, 21, 22 and 23.

The originally described species of *M. rainai* was named as *Rhabditis rainai* isolated by Osbrink and Carta (2005) from New Orleans, USA. Later Sudhaus (2011) revised *R. rainai* into *M. rainai* along with few other species. *M. rainai* (Isolate 2) differed from species in the *Rhabditis* (*Oscheius*) *dolichura* group (Sudhaus and Hooper, 1994) which have nine bursal rays and spicules with swollen, uncurved distal tip. *M. rainai* (Isolate 2) differed from leptoderan-tailed, nine-ray species in the *Rhabditis* (*Oscheius*) *insectivora*-group (Sudhaus and Hooper, 1994) in having a peloderan tail with eight rays, expansile rectum and spicule lacking a distal hook. *M. silvatica* has a pre rectum, but differs from *M. rainai* (Isolate 2) in having a median pharyngeal bulb, three stomatal teeth, nine bursal rays with leptoderan male tail, lower 'b' ratio (4.5 *vs* 10.8) and smaller spicule axis length (34.00 *vs* 39.63µm). *M. rainai* differed from *M. blumi* Sudhaus and from *M. adenobia* Poinar in the hermaphroditic condition with rare males induced by starvation, a peloderan rather than leptoderan tail, spicule not sabre-like with gentle neck curvature. Compared to

M. adenobia, M. rainai (Isolate 2) had a wider hermaphrodite body (86.13µm (79-98) µm *vs* 55 (46-69) µm), and lower hermaphrodite 'a' value (12.51 (11.48-13.45) *vs* 20.8 (19-24)). *M. rainai* (Isolate 2) males had a shorter body length than *M. adenobia* (696.60 (644-860) µm *vs* 926 (768-1248) µm). Compared to *M. blumi* (Sudhaus, 1974), *M. rainai* (Isolate 2) had a smaller pharynx length (Hermaphrodite – 198.5 (162-208) µm *vs* 246 (244-298) µm ; Male- 138 (134-156) µm vs (226-276) µm), smaller hermaphrodite 'a' value (12.51 (11.48-13.45) µm *vs* 26.4 (24-28) µm), shorter spicule axis length (44.10 (40- 51) µm vs 54 (48-63) µm). The morphometric characters of *M. rainai* (Isolate 2) compared with *M. rainai* (Fiji population) showed similarities in hermaphrodite (1374.26±63.00µm vs 1245±233µm) and male (696.60±73.89µm vs739±37µm) body lengths (Table 18). The oesophageal length of males (138.00±5.33µm vs 144±4µm) of both strains were also similar. Morphological features of spicules (39.63±3.59µm vs 45±3.6µm) varied in both strains though morphologically they were similar. Male tail of *M. rainai* (Isolate 2) (28.60±5.10µm vs 36±2µm), was shorter compared to *M. rainai* (Fiji population). Both the *M. rainai* strains had same range of values of ratios a, b and c.

The Isolate 2 obtained from Mylom area in this study showed similarity to *M. rainai* in morphometric measurements. So to confirm the identity molecular characterization of Isolate 2 was done by amplifying the internal transcribed region of the isolate using TTGATTACGTCCCTGCCCTTT as forward primer and TTTCACTCGCCGTTACTAAGG as reverse primer (Vrain *et al.*, 1992). Blast details of the most matching sequence homology in NCBI data base revealed the identity of Isolate obtained from Mylom as *M. rainai*.

The Isolate 3 was obtained from Kainidi, Alappuzha was identified as *Rhabditis* sp. The characteristics of this native isolate were similar to the males and females of Isolate 2 (*M. rainai*) obtained from Mylom, Kottarakara. Most of morphological characters were similar with some differing characters. Infective Juveniles had a thin elongate body with a closed stoma. Males were J shaped

(804.67±46.34µm) and females (1337.14±47.18µm) were elongate. The lips were unfused with funnel shaped stoma. They had a rhabditoid oesophagous with a well-developed butterfly valve (females- 208.14±5.64µm; males- 84.71±5.35µm). Beneath the basal bulb, a distinct cardia was visible (Plate 30). Excretory pore was seen anterior to the basal bulb (females- 139.25±5.50µm; males- 71.67±4.72µm). The vulval lips were slightly protruding near to the anal slit (Plate 32).The distinguishing features among the strains were a ventrally curved tail in males (29.71±4.86µm), spicules (28.88±4.26µm) with a manubrium which was not round and oesophageal glands which were not lobed unlike *M. rainai* (Isolate 2). The above mentioned characters are shown in Plates 24, 25 and 26.

Rhabditis sp. (Isolate 3) was similar to species in the *Rhabditis* (*Oscheius*) *insectivora* group as they had nine bursal rays and swollen spicules with a leptoderan tail. On comparing *M. silvatica* with *Rhabditis* sp. lower 'b' ratio (9.72 *vs* 10.8) was observed in *Rhabditis* sp. and smaller spicule axis length (28.88 *vs* 39.63µm). *Rhabditis* sp. (Isolate 3) differed from *M. rainai* as hermaphroditic generation was absent in them. Compared to *M. adenobia, Rhabditis* sp. males had a shorter body length than *M. adenobia* (804 (741-862) µm *vs* 926 (768-1248) µm). Compared to *M. blumi, Rhabditis* sp. (Isolate 3) had a smaller pharynx length (Females-208.14 (202-216) µm vs 266 (212-298) µm; Male- 84.71 (78-94) µm vs (226-276) µm) and shorter spicule axis length (28.88 (22-37) µm vs 54 (48-63) µm).

5.2 SCREENING OF EPN ISOLATES FOR INSECTICIDAL PROPERTY UNDER *IN VITRO* CONDITIONS.

The three native EPNs isolates obtained through random soil sampling were subjected to screening for testing their pathogenicity against test insects *viz.*,termites, aphids, tobacco caterpillar and pseudostem weevil. The test insects were treated with 300 IJs of native EPN isolates and their mortality percentage was recorded 24 hours

after treatment (HAT). The emergence of IJs from infected cadavers and change in colour of the cadavers were observed.

The data presented in 4.2 revealed that among the three native strains, Isolate 2 obtained from Mylom area, *M. rainai* showed the highest mortality (87.99 per cent) followed by Isolate 1 obtained from Vellayani area, *Steinernema* sp. (86.00 per cent) and Isolate 3 obtained from Kainidi area, *Rhabditis* sp. (76.00 per cent) at 24 HAT with 300 IJs/termite. The emergence of IJs was found highest in *M. rainai* (Isolate 2) ($2.0x10^3$) compared to other two isolates and cadaver infected with *M. rainai* (Isolate 2) was brownish in colour as mentioned in Plate 9. Results of the study revealed that mortality of termites was significantly influenced by nematode species. Many researchers have reported the efficacy of *Steinernema* and *Rhabditis* strains against termites. *M. rainai* was isolated from the gut and head of *Coptotermes formosanus* (Osbrink and Carta, 2005). Razia and Sivaramakrishnan (2016) reported *S. siamkayai* caused 84.00 per cent mortality of termites with a concentration of 300 IJs/termite at 24 HAT. They also reported emergence of 1800 IJs of *S. pakistanense* from termite cadaver and the cadaver had a brick red colour.

In the case of aphids, *M. rainai* (Isolate 2) recorded highest per cent mortality (86.00 per cent) followed by *Steinernema* sp. (Isolate 1) (81.74 per cent) and *Rhabditis* sp. (Isolate 3) (71.99 per cent) with 300 IJs/aphids at 24 HAT. *M. rainai* (Isolate 2) showed the highest emergence of IJs ($1.2.x10^3$) compared to *Steinernema* sp. (Isolate 1) ($0.9x10^3$) and *Rhabditis* sp. (Isolate 3) ($0.2x10^3$). The difference in aphid mortality observed in this study may be attributed to the host preference by nematodes, nematode's compatibility with host and vulnerability of insect pests to specific nematode infection (Shapiro-Ilan and Cottrell, 2005). Maketon *et al.* (2011) reported that *H. indica* was able to penetrate and kill *Aphis gossypii* but reproduced poorly.

M. rainai (Isolate 2) recorded the highest mortality (29.99 per cent) followed by *Steinernema* sp. (Isolate 1) (19.99 per cent) and *Rhabditis* sp. (Isolate 3) (19.99 per cent) with 300 IJs per larvae at 24 HAT in tobacco caterpillar. *M. rainai* (Isolate 2) showed the highest emergence of IJs ($3.5x10^5$) compared to *Steinernema* sp. (Isolate 1) ($2.7x10^5$) and *Rhabditis* sp. (Isolate 3) ($0.9x10^5$). The cadavers infected with *M. rainai* (Isolate 2) had pinkish colour whereas cadavers infected with *Steinernema* sp. (Isolate 1) and *Rhabditis* sp. (Isolate 3) not showed any colour change (as mentioned in Plate 13. The mortality percentage observed in this study was different from the findings of Gupta (2003). He reported a per cent mortality of 40-60 in tobacco caterpillars treated with 50-100 IJs/larva applied as foliar spray. The results of emergence of IJs from tobacco caterpillar obtained in the study were similar to the observations of Pervez and Ali (2009). They observed that *in vivo* production of IJs on *S. litura* showed the highest yield in *S. mushtaqi* ($0.79x10^5$ IJs/cadaver) followed by *S. seemae* ($0.72x10^5$ IJs/cadaver), *S. carpocapsae* ($0.67x10^5$ IJs/cadaver) and *S. masoodi* ($0.51x10^5$ IJs/cadaver). There was no report on colour change of the tobacco caterpillar infected with EPN. However *G. mellonela* infected with Steinernematids showed cream or ochre-grey colour whereas Heterorhabditids showed red or burgundy colour (Yan *et al.*, 2016).

Results of screening of three native isolates against pseudostem weevil grubs revealed that *Steinernema* sp. (Isolate 1) and *M. rainai* (Isolate 2) at 300 IJs/pseudostem weevil grub showed a mortality percentage of 9.99 whereas no mortality was observed in *Rhabditis* sp. (Isolate 3) at 24 HAT. The emergence of IJs was found highest in Isolate 2 ($3.5x10^5$) compared to *Steinernema* sp. (Isolate 1) ($1.5 x10^5$) and *Rhabditis* sp. (Isolate 3) ($0.3x10^5$). *Steinernema* sp. (Isolate 1) and *M. rainai* (Isolate 2) showed a discolouration of creamish and reddish brown respectively whereas *Rhabditis* sp. (Isolate 3) not showed any colour change (as mentioned in Plate 15. The results of the present study were in line with the findings

of Padmanabhan *et al.* (2002). They reported that *O. longicollis* third-instar grubs treated with an inoculum level of 10-70 IJs and 80-100 IJs/grub of *H. indica* caused 33.3 and 66.6 per cent mortality respectively at 72 HAT. There was no report on the emergence of IJs from a cadaver and colour change of the cadaver in case of pseudostem weevil. However Bharathi and Mohite (2013) reported a discolouration in cadaver of *Leucopholis lepidophora* due to the infection by *H. indica*.

In the present study, among the three isolates, *M. rainai* (Isolate 2) at 300IJs performed best in killing the termites, aphids, tobacco caterpillar and pseudostem weevil grubs at 24 HAT. Selection of appropriate EPNs with virulence and host searching ability is essential for successful and effective biological control. Bio control potential of *M. rainai* against termites, aphids, tobacco caterpillar and pseudostem weevil is reported first time in this study.

5.3 PATHOGENICITY OF EPN

Pathogenicity of native EPN isolates termites, aphids, tobacco caterpillar and pseudostem weevil were assessed with inoculum levels of 10, 50, 100 and 200 IJs at different time periods *viz.*, 24, 36, 48, 60 and 72 HAT. The results presented in Tables 4-15 showed statistically significant variation between different levels of isolates on mortality of test insects.

Highest mortality of termites was recorded in *Steinernema* sp. (Isolate 1) and *M. rainai* (Isolate 2) (cent per cent) with 100 and 200 IJs per termite at 48 HAT which was as effective as Chemical, Chlorpyriphos 25EC (Fig 1). *Rhabditis* sp. (Isolate 3) required an exposure period of 60 h and inoculum level of 200 IJs/termite for attaining the same mortality as *Steinernema* sp. (Isolate 1) and *M. rainai* (Isolate 2). *M. rainai* (Isolate 2) recorded the highest mortality (cent per cent) with 50 IJs per termite followed by *Steinernema* sp. (Isolate 1) (99.08 per cent) and *Rhabditis* sp. (Isolate 3) (73.53 per cent) at 60 HAT (Fig 2). *Steinernema* sp. (Isolate 1) recorded cent per cent mortality with 50 IJs per termite at 72 HAT whereas *Rhabditis* sp.

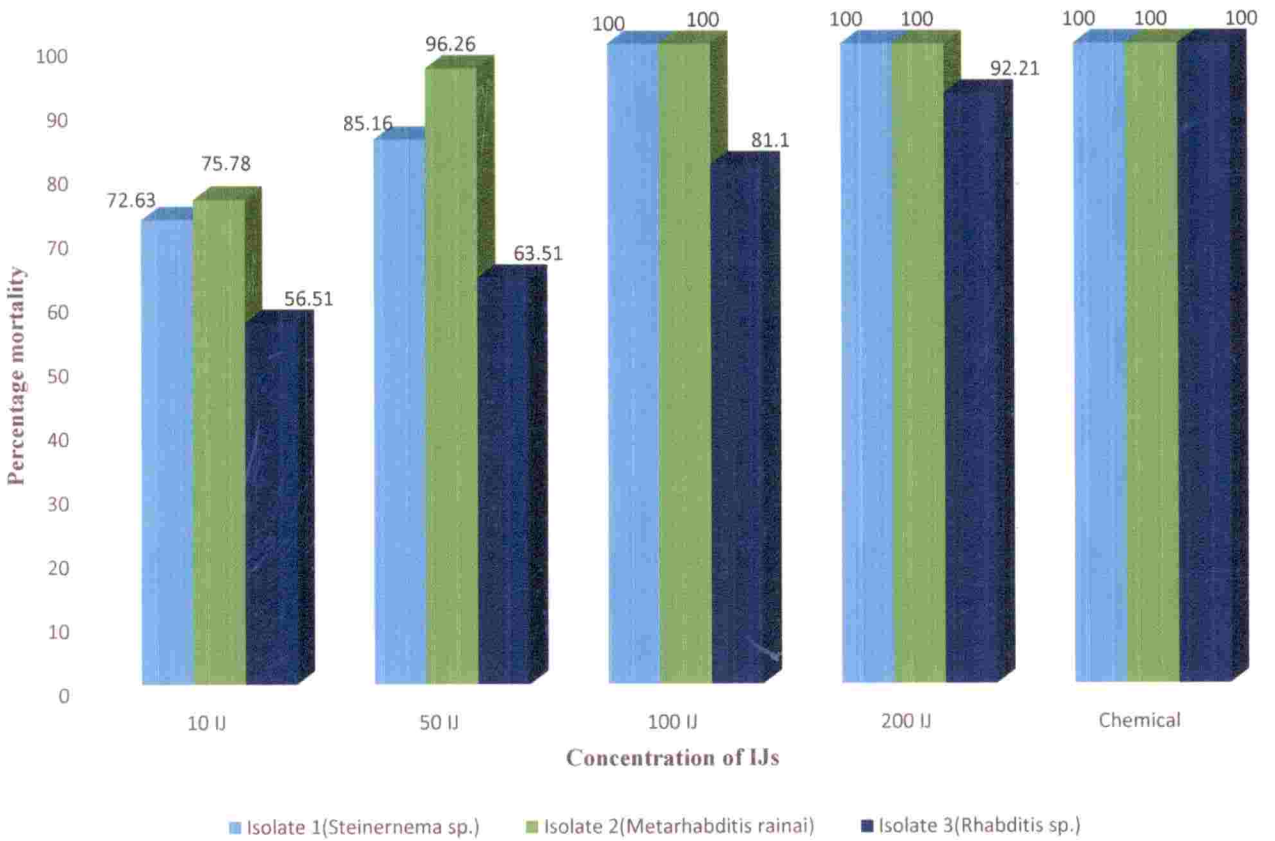


Fig. 1 Effect of different levels of native isolates on the mortality of termites at 48 HAT under laboratory conditions

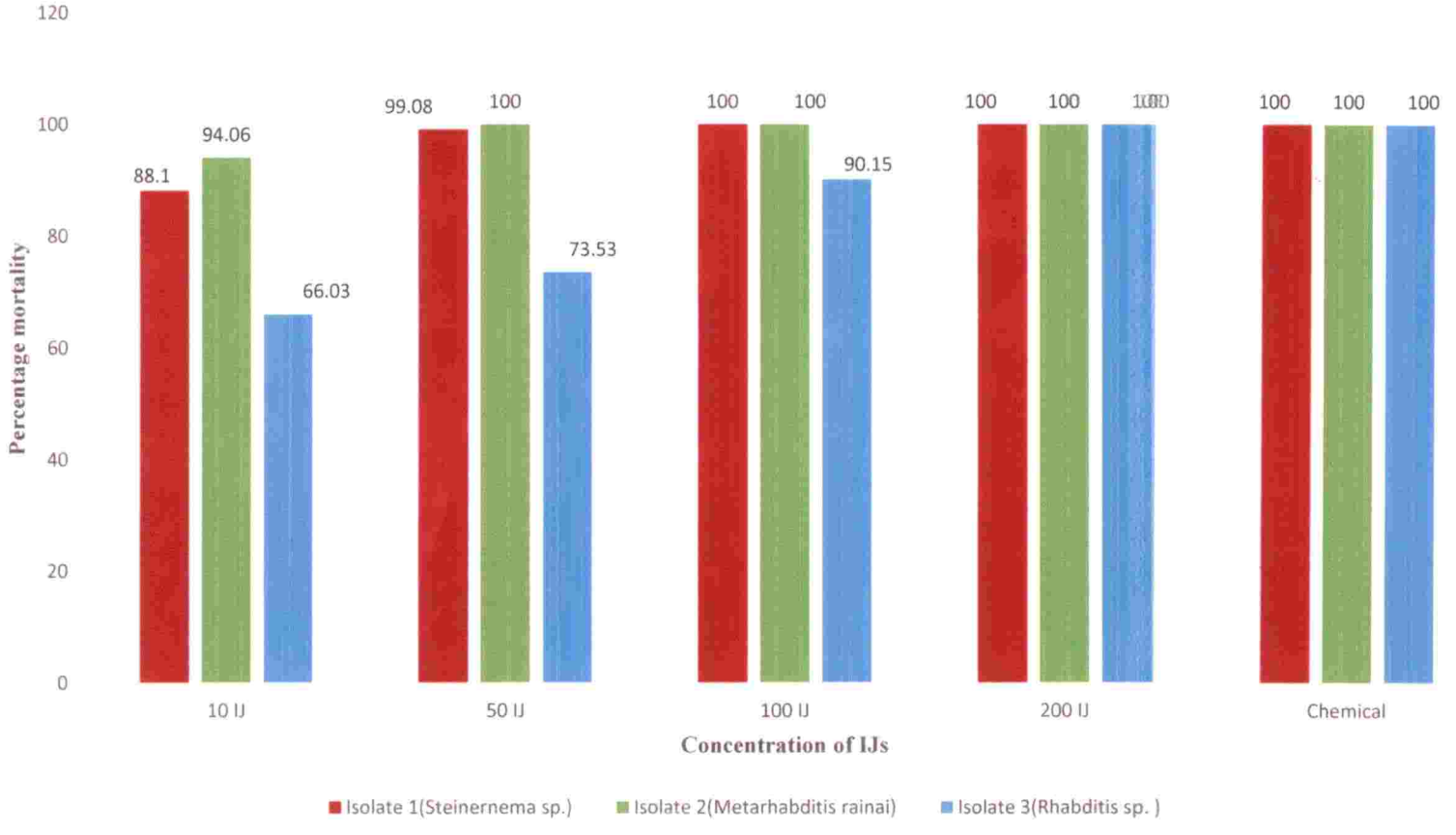


Fig. 2 Effect of different levels of native isolates on the mortality of termites at 60 HAT under laboratory conditions

(Isolate 3) recorded only 81.53 per cent mortality at same inoculum level and time period. The observation clearly showed that an increase in the inoculum level, decreased the time period of termite mortality. The efficacy of different isolates of EPN for controlling a particular insect pest may differ and is influenced by the rate of IJ penetration into the insect, the virulence and time it takes to release the symbiotic bacteria (Glazer and Navon, 1990). The result obtained in the present study was superior over the findings of Wang *et al.* (2002) in which *S. carpocapse* and *S. riobrave* caused significant mortality of *C. formosanus* only after 8 days of treatment with 400 IJs/termite. The results of the present investigation were in agreement with the findings of Zadji *et al.* (2014) and Razia *et al.* (2016). An experiment conducted by Zadji *et al.* (2014) recorded 96.3 per cent mortality of termites, *Macrotermes bellicosus* with *H. indica* (50 IJs/ termites) after 48 hours of treatment. Another study conducted by Razia *et al.* (2016) recorded cent per cent mortality of *R. flavipes* with 500 and 700 IJs/ml of *S. siamkayai* and *S. pakistanense* respectively within 48 hours of exposure. The present study reveals the bio efficacy of *Rhabditis* sp. against termites for the first time.

Steinernema sp. (Isolate 1) and *M. rainai* (Isolate 2) at 200 IJs recorded cent percent mortality of aphids at 48 HAT and it was statistically on par with chemical (Dimethoate 30EC) followed by *Rhabditis* sp. (Isolate 3) (91.87 per cent). *M. rainai* (Isolate 2) at 100IJs/aphid recorded highest mortality (cent per cent) than *Steinernema* sp. (Isolate 1) (98.54 per cent) at 48 HAT (Fig 3). But *Steinernema* sp. (Isolate 1) needed an inoculum level of 200 IJs/aphid to get cent per cent mortality at 48 HAT. *Rhabditis* sp. (Isolate 3) recorded cent per cent mortality at 60 HAT with 200 IJs/aphid. Highest mortality with 50 IJs at 60 HAT was observed with *M. rainai* (Isolate 2) (99.87 per cent) followed by *Steinernema* sp. (Isolate 1) (91.58 per cent) and *Rhabditis* sp. (Isolate 3) (67.04 per cent) (Fig 4). At the end of 72 hours, 50 IJs of *Steinernema* sp. (Isolate 1) and *M. rainai* (Isolate 2) recorded cent per cent mortality whereas *Rhabditis* sp. (Isolate 3) showed only 82.58 per cent mortality. Many works

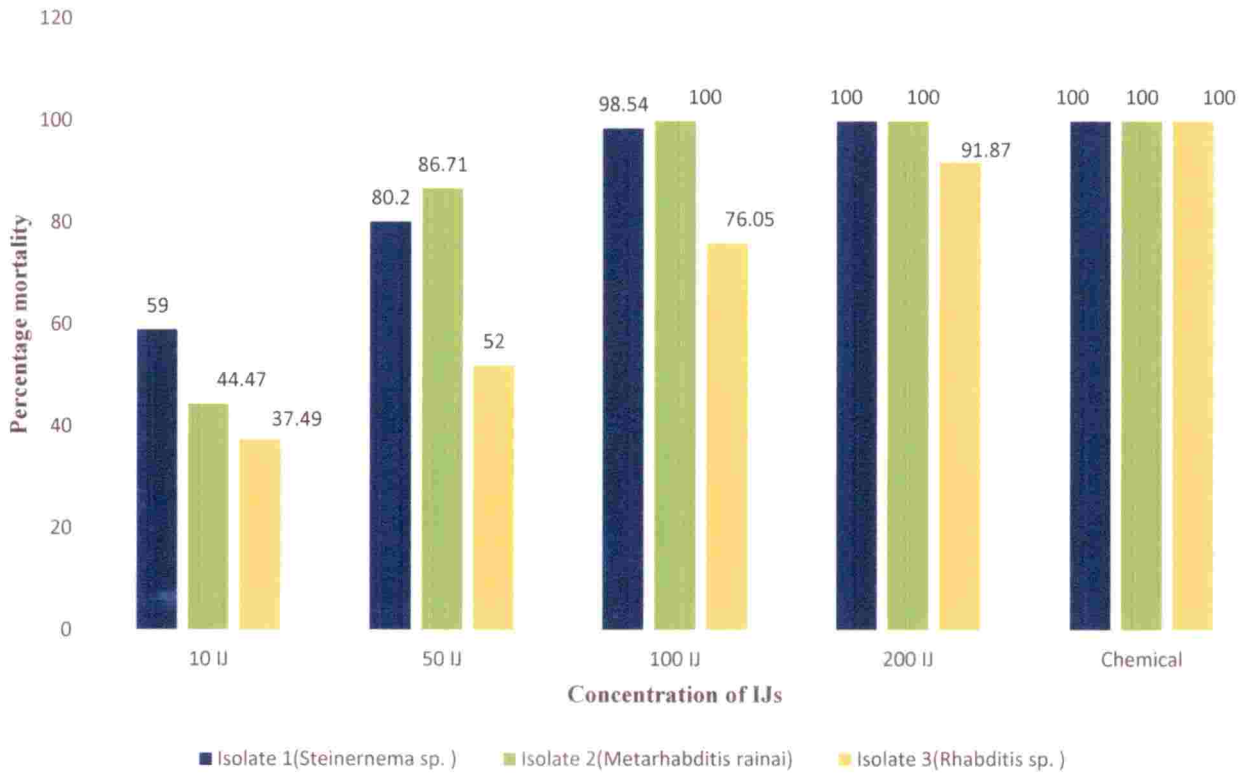


Fig. 3 Effect of different levels of native isolates on the mortality of aphid at 48 HAT under laboratory conditions

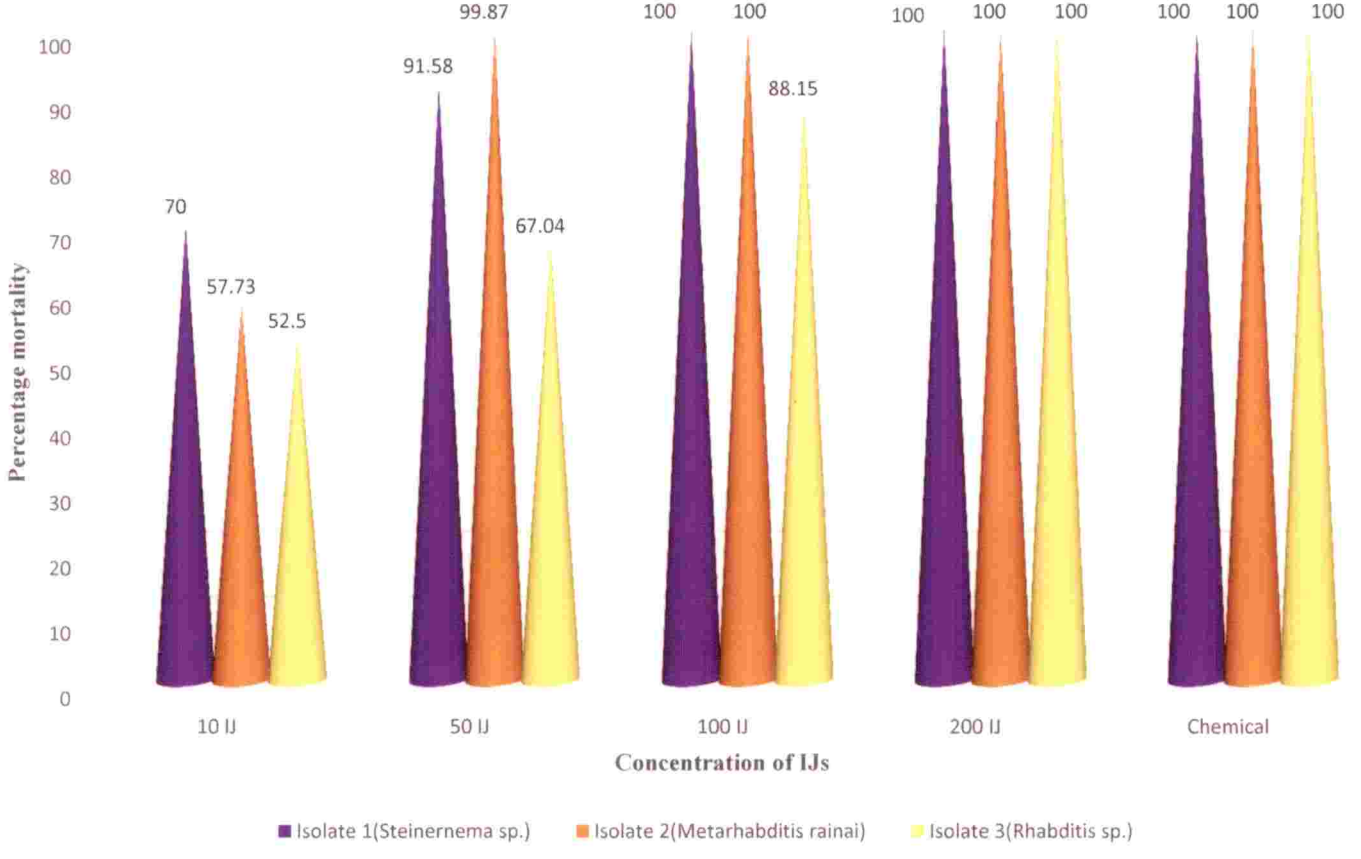


Fig. 4 Effect of different levels of native isolates on the mortality of aphids at 60 HAT under laboratory conditions

have been conducted using *Steinernema* and *Heterorhabditis* against various homopteran insect pests under controlled environment by several researchers but results were less effective (Stuart *et al.*, 1997). Conversely, the present study reveals the potential of *Rhabditis* sp. in causing mortality of aphids with lowest inoculum levels and period of exposure. The results obtained with *Steinernema* sp. (Isolate 1) agree with the observations of Kumar and Ganguly (2011). They conducted a leaf disc assay and reported that *S. thermophilum* (New Delhi strain) caused a mortality percentage of 66-83 in *Aphis gossypii* when treated with inoculum levels ranging from 50-500 IJs/ml after 72 HAT. The finding of the present study in aphids was also in agreement with Sheela *et al.* (2006) who reported that a native EPN strain from Kerala recorded cent per cent mortality of rice bug at lowest inoculum level of 10 IJs.

Among the EPN isolates, *M. rainai* (Isolate 2) at 200 IJs recorded the highest mortality of tobacco caterpillar (99.35 per cent) followed by *Steinernema* sp. (Isolate 1) (85.36 per cent) and *Rhabditis* sp. (Isolate 3) (85.35 per cent) at 72 HAT. When the concentration of IJs was reduced to 100 IJs per larvae, highest mortality was recorded by *M. rainai* (Isolate 2) with 92.53 per cent mortality followed by *Rhabditis* sp. (Isolate 3) (80.00 per cent) and *Steinernema* sp. (Isolate 1) (73.20) at 72 HAT (Fig 6). Mortality percentage at 60 HAT with 200 IJs was highest in *M. rainai* (Isolate 2) (80.52) followed by *Steinernema* sp. (Isolate 1) (75.17) and lowest in *Rhabditis* sp. (Fig 5) (Isolate 3) (65.08). Percentage mortality was found directly proportional to concentration of IJs and period of exposure. Several workers reported the efficacy of EPNs against *S. litura*. Rajkumar *et al.* (2002) reported that sand column bioassay tests of *Heterorhabditis* sp. (HUDP-1 strain) against *S. litura* and recorded mortality per cent ranging from 16.7 to 88.9 when treated with 25, 50, 75, 100, 125 and 150 IJs/caterpillar. Gupta *et al.* (2008) reported 100 per cent mortality of 3-5 instar larvae of *S.litura* after 96 hours of treatment in laboratory conditions by a local isolate of *S. carpocapsae*. Yadav *et al.* (2017) reported 100 per cent mortality of *S. litura* at an inoculum level of 400 IJs of *S. carpocapsae* produced on animal

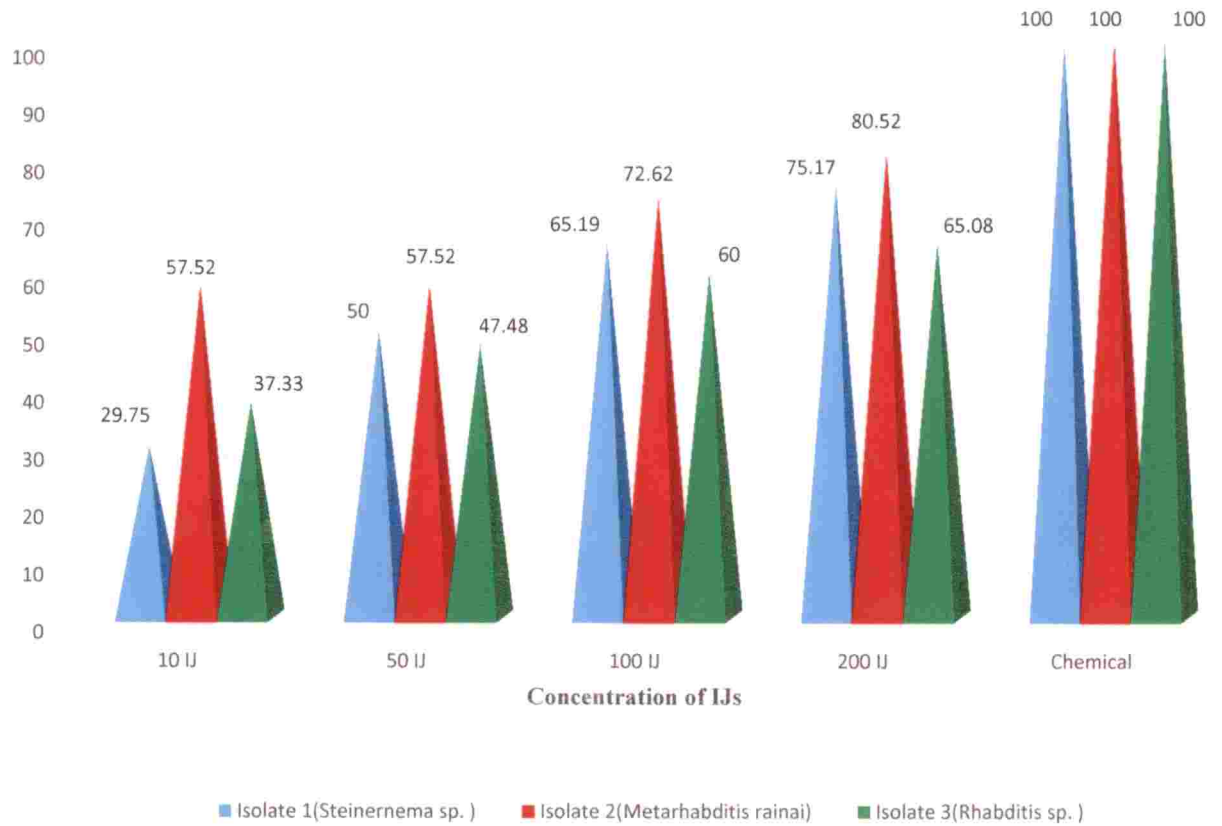


Fig. 5 Effect of different levels of native isolates on the mortality of tobacco caterpillar at 60 HAT under laboratory conditions

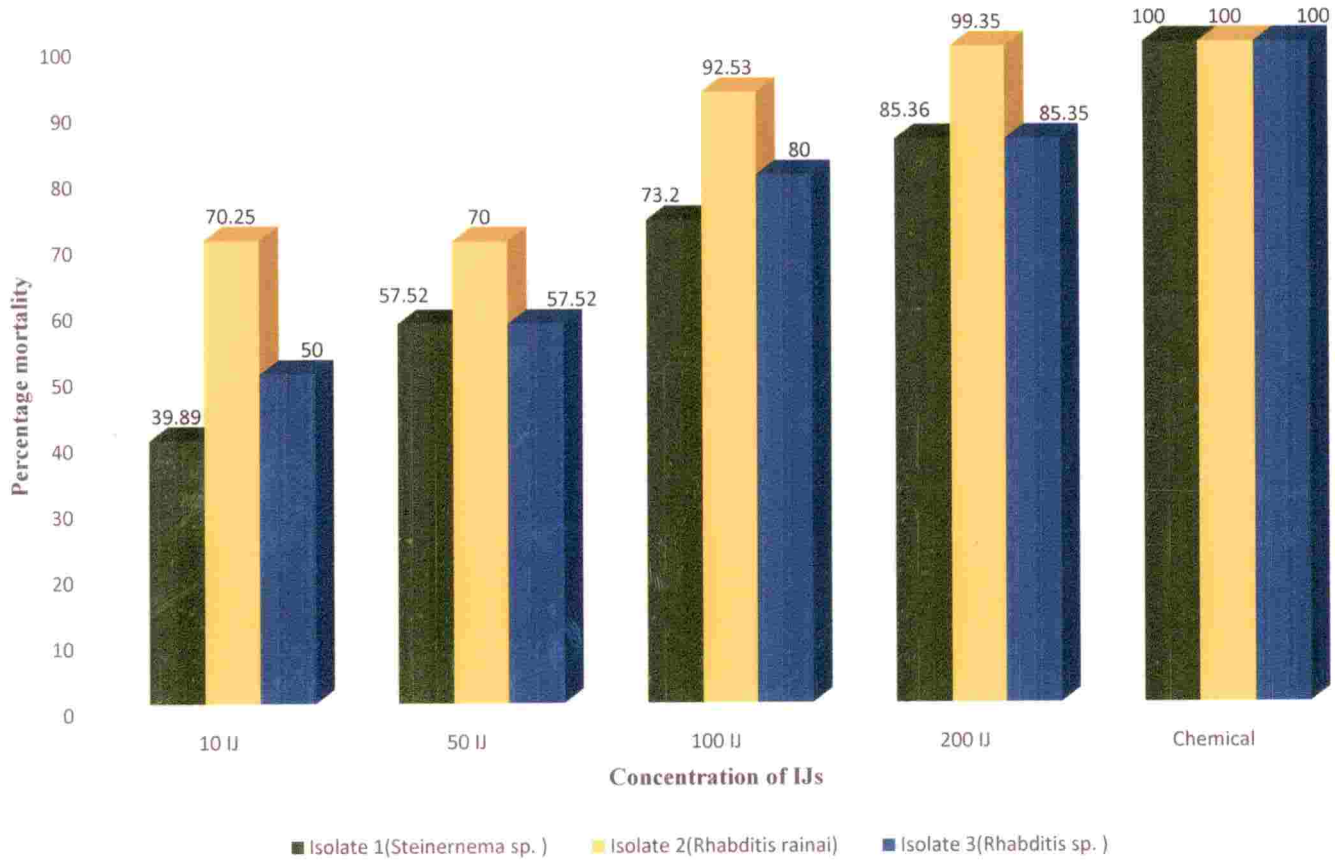


Fig. 6 Effect of different levels of native isolates on the mortality of tobacco caterpillar at 72 HAT under laboratory conditions

protein based media at 96 hours after exposure. But the present study is pioneer in testing the pathogenicity of *Rhabditis* sp. against *S. litura*. However, Padmakumari *et al.* (2007) reported *Rhabditis* sp. causing mortality of egg mass and neonate larvae of rice yellow stem borer at 500 IJs/larvae at 31 h.

On comparing native EPN isolates, the mortality of pseudostem weevil grubs was highest in *M. rainai* (Isolate 2) with 200 IJs/grub at 72 HAT (62.66 per cent). 200 IJs of *Steinernema* sp. (Isolate 1) and *Rhabditis* sp. (Isolate 3) recorded only 50.00 and 37.44 per cent at the same time period (Fig. 5). *M. rainai* (Isolate 2) recorded 47.49 per cent mortality with 100 IJs per grub at 72 HAT whereas *Steinernema* sp. (Isolate 1) and *Rhabditis* sp. (Isolate 3) recorded only 44.97 and 29.75 per cent mortality respectively at the same level and period of exposure. This finding clearly highlights a reduced mortality percentage of pseudostem weevil grubs with IJs which might be due to restricted movement of grubs inside the pseudostem. Chemical showed highest mortality of *O. longicollis* grubs at 72 HAT. The results of this study were in line with the findings of Padmanabhan *et al.* (2002) who reported that *O. longicollis* third-instar grubs treated with inoculum levels of 10-70 and 80-100 IJs/grub caused 33.3 and 66.6 per cent mortality respectively after 72 hours of treatment. A similar work was undertaken by Mwaitulo *et al.* (2011) using native strains belonging to genera *Steinernema* and *Heterorhabditis* with inoculum levels *viz.*, 100, 500 and 1000 IJs/grub in a cylindrical piece of pseudostem. They reported that with increase in dosage of EPNs the mortality of *O. longicollis* grubs increased. *Rhabditis* sp. as a potential EPN was revealed for the first time through the present study.

The present study highlights the biocontrol potential of three native isolates against the test insects at different inoculum levels and exposure periods. Nematode-bacteria complex plays significant role in insect mortality. Due to difference in nematode species, their associated bacteria also reacted differently in different types of insects. Rates of nematode infection and insect mortality is dose dependent and

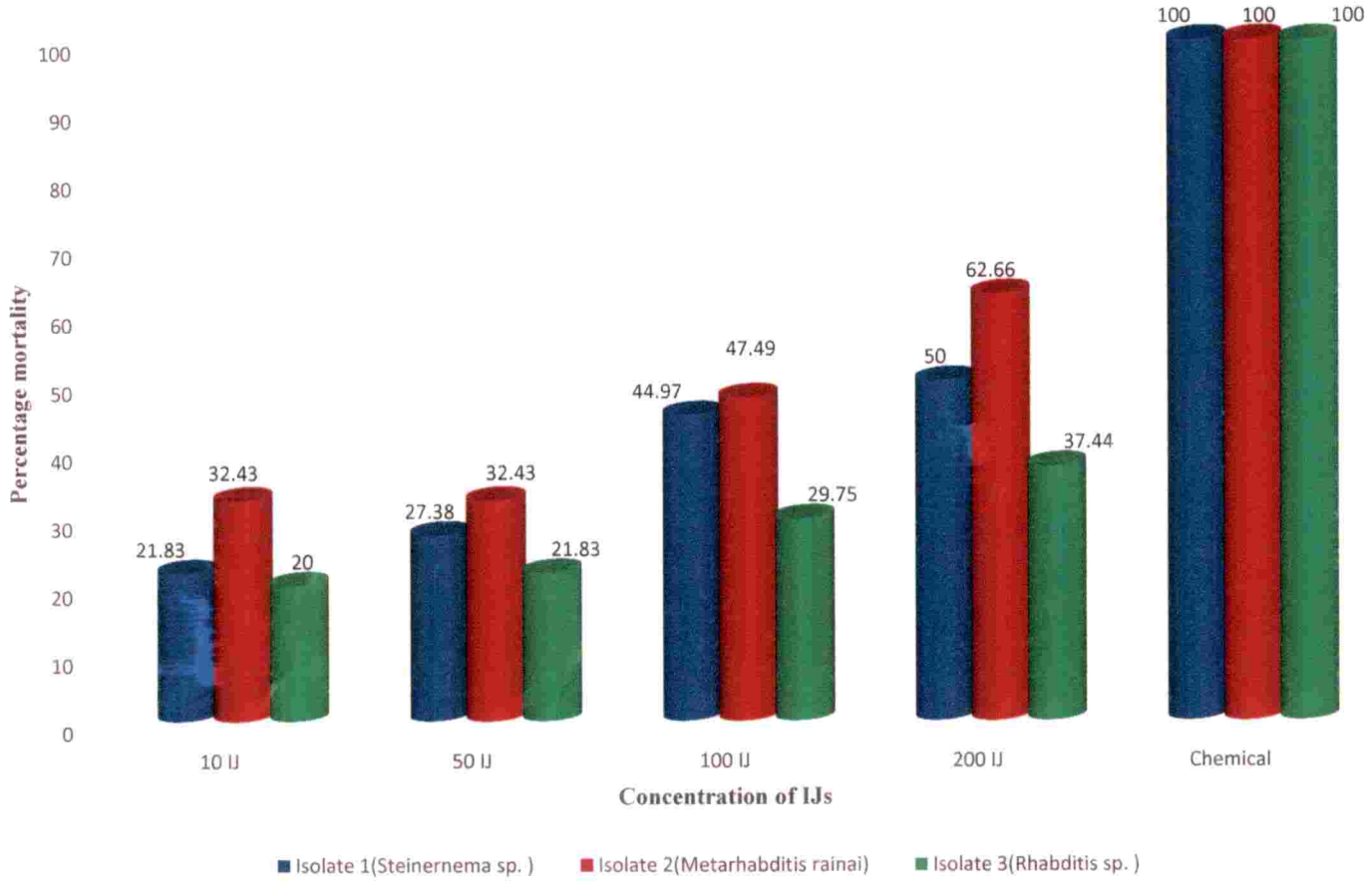


Fig.7 Effect of different levels of native isolates on the mortality of pseudostem weevil grubs at 72 HAT under laboratory conditions

varies depending on the type of substrate, insect and experimental conditions. Based on the findings it can be stated that *M. rainai* can be exploited for the sustainable management of termites, aphids, tobacco caterpillar and pseudostem weevil due to low inoculum requirement for killing the pests and high efficacy.

Summary

6. SUMMARY

The study entitled "Pathogenicity of indigenous entomopathogenic nematodes against select insect pests" was conducted in Department of Nematology, College of Agriculture, Vellayani during 2017-19. The main objective was to isolate indigenous entomopathogenic nematodes (EPN) and to determine their potential in causing mortality of different groups of pests.

A survey was conducted in different districts of Kerala *viz.*, Thiruvanathapuram, Kollam, Pathanamthitta and Alappuzha for isolation of indigenous EPNs from different crop habitats. A total of forty soil samples were collected from the rhizosphere of vegetables, banana and coconut through random sampling. Three native EPN strains were isolated from the collected soil sample through "insect baiting technique". Isolate 1 was obtained from soil samples collected from the rhizosphere region of cowpea plants raised in College of Agriculture, Vellayani. Isolate 2 was obtained from samples collected from tomato plant grown in a multicropped field in Mylom, Kottarakara (Kollam). Isolate 3 was obtained from the banana rhizosphere in Kainidi area of Alappuzha district. The native EPN strains were mass multiplied in corcyra larvae and were stored separately in tissue culture flasks which were kept in BOD incubator at 15°C. All the three isolates were screened for their insecticidal property against termites, aphids, tobacco caterpillars and pseudostem weevils under *in vitro* condition.

Results obtained in preliminary screening of native strains against test insects using 300 IJs/ insect showed that Isolate 2 recorded the maximum emergence of IJs from termites ($2x10^3$IJs/adult), aphids ($1.2x10^3$IJs/adult), tobacco caterpillar ($3.5x10^5$IJs/larvae) and pseudostem weevil ($3.5x10^5$IJs/grub). Isolate 2 at 300 IJs showed mortality percentage of 87.99, 86.00, 29.99 and 9.99 against termite, aphids, tobacco caterpillar and pseudostem weevil respectively at 24 HAT. Isolate 1 and 3 showed mortality of 86.00 and 76.00 per cent in termites and 81.74 and 71.99 per

cent in aphids respectively at 24 HAT. No appreciable mortality was shown by Isolate 1 and 3 in tobacco caterpillar and pseudostem weevil grub.

Infection of Isolate 2 resulted in brown, pink and reddish brown discolouration in cadavers of termites, tobacco caterpillars and pseudostem weevil grubs respectively. Isolate 1 showed a creamish discolouration in pseudostem weevil grubs. Isolate 3 did not show any colour change in cadavers of any test insect. No discolouration was observed in aphids by any of the native isolates.

Based on results of preliminary screening, Isolate 1, 2 and 3 were tested for their pathogenicity against test insects with 10, 50, 100 and 200 IJs/ insect at 24, 36, 48, 60 and 72 HAT under *in vitro* condition. In termites, Isolate 1and 2 showed maximum mortality (cent per cent) with 100 and 200 IJs at 48 HAT and effect of these two native isolates was statistically on par with the chemical. All the inoculum levels of Isolate 1 and 2 recorded cent per cent mortality at 72 HAT. Isolate 3 showed cent per cent mortality of termites with 200 IJs at 72 and 60 HAT respectively.

In the case of aphids, Isolate 2 recorded maximum mortality (cent per cent) with 100 and 200 IJs at 48 HAT and were statistically on par with the chemical. All the inoculum levels of Isolate 2 except 10 IJs showed cent per cent mortality at 72 HAT. Even 200 IJs of Isolate 1 recorded cent per cent mortality at 48 HAT. Isolate 3 showed cent per cent mortality with 200 IJs at 60 HAT.

Tobacco caterpillar was found to be less susceptible to lower doses of native EPN strains. Chemical showed cent per cent mortality within 24 h of exposure. However, 200 IJs of Isolate 2 showed a mortality of 80.52 and 99.35 per cent at 60 and 72 HAT respectively. Isolate 1 recorded 75.17 and 85.36 per cent mortality with 200 IJs at 60 and 72 HAT. Isolate 3 recorded the least mortality among the isolates at 200 IJs with 65.08 and 85.35 per cent at 60 and 72 HAT respectively.

Among the test insects, the pseudostem weevil grubs recorded the least mortality percentage when treated with native EPNs. Chemical showed cent per cent mortality at 48 HAT. 100 and 200 IJs of Isolate 2 recorded 47.49 and 62.66 per cent mortality at 72 HAT. Isolate 1 showed a mortality percentage of 44.97 and 50.00 with 100 and 200 IJs at 72 HAT. Isolate 3 at 100 and 200 IJ level recorded 29.75 and 37.44 per cent mortality at 72 HAT. No appreciable mortality percentage was observed with lower inoculum levels (50 and 10 IJs) in any of the native isolates.

The native EPN strains were identified based on the morphological characters and morphometric measurements. Isolate 1 was identified as *Steinernema* sp. The IJs had an elongate and thin body which retained the second stage cuticle. They had closed mouth opening. Females were C shaped and males were J shaped. The anterior region of both males and females had rounded lip with short stoma. The excretory pore (154.00±5.57µm) was seen anterior to the nerve ring in both males and females. The oesophagous of both males (144.40±4.14µm) and females (165.60±6.74µm) was muscular with cylindrical procorpus, slightly enlarged metacorpus, short and narrow isthmus surrounded by a nerve ring and rounded basal bulb. Vulva was seen as a transverse slit situated on a protuberance and was sub medially located which was a typical character of *Steinernema* females. The males had symmetrical, slightly curved and paired spicules (44.10±4.46µm). Beneath the spicule, a boat shaped gubernaculum (23.70±2.98µm)was present. The females had a short and conoid tail (54.10±5.26µm)with a pointed tip. The male tail (35.80±5.20µm) was conoid with a mucron and withoutbursa.

Based on the morphometric and molecular analysis, Isolate 2 was identified as *Metarhabditis rainai*. IJs had a narrow and elongated body and they have not retainedtheir 2nd stage cuticle. The tail of IJs was long and pointed and covered with a sheath. Hermaphrodites obtained in the first generation had a C shaped body unlike that of males and females. Females had a long and elongated body whereas males had a J shaped body. The stoma was tubular/ funnel shaped in hermaphrodites, males and

females. Hermaphrodites had well cuticularized cheilorhabdions unlike males and females in which cuticularized cheilorhabdions were absent. Hermaphrodite had a cylindrical corpus (198.50±8.44µm) throughout whereas the males (138.00±5.33µm) and females (215.78±10.60µm) had a typical "Rhabditoid oesophagous" with a cylindrical procorpus, undifferentiated metacorpus, distinct isthmus and pyriform shaped basal bulb with well-developed butterfly valve. The oesophageal glands were lobed protruding into the intestine. Hermaphrodites had a conoid tail (98.70±9.03µm) with post-anal swelling and pointed terminus. Females had a filiform tail (166.50±12.11µm) whereas males had a short, conoid and pointed tail. Vulva was a tranverse slit with slightly protruding vulval lips and was seen near to the anal slit. The male tail (28.60±5.10µm) had a peloderan bursa with eight genital papillae. The spicules (39.63±3.59µm) were paired and asymmetrical and slightly curved ventrally. Gubernaculum (28.00±3.86µm) was slightly curved ventrally and had a length more than half the spicule length.

Isolate 3 was identified as *Rhabditis* sp. based on the morphological characters and morphometric measurements. Isolate 3 was similar to the males and females of Isolate 2 obtained. Most of morphological characters were similar with some differing characters. The distinguishing features among the strains were a ventrally curved tail, spicules with a manubrium which was not round and oesophageal glands were not lobed.

Based on the pathogenicity studies, Isolate 2 is identified as the most potent EPN strain which caused maximum mortality in termites, aphids, tobacco caterpillar and pseudostem weevil. Molecular characterization of Isolate 2 revealed it as *Metarhabditis rainai*.

Result of the study revealed the biocontrol potential of native strains of EPN against different test insects. Among the three native isolates, Isolate 2 was identified as *Metarhabditis rainai* which proved to be the most potent strain and can be

recommended in IPM programmes without any harmful effect on the environment. An effort needs to be directed towards formulating the strain so as to improve its efficiency and shelf life.

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PATHOGENICITY OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES AGAINST SELECT INSECT PESTS

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Abstract of the thesis

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ABSTRACT

An investigation entitled "Pathogenicity of indigenous entomopathogenic nematodes against select insect pests" was carried out at the Department of Nematology, College of Agriculture, Vellayani during 2017-19. The main objectives were to identify indigenous entomopathogenic nematodes and evaluate their pathogenicity to termites, lepidopteran and coleopteran pests.

Survey was conducted in Thiruvanathapuram, Kollam, Pathanamthitta and Alappuzha districts during 2017-18 and a total of forty soil samples were collected from the rhizosphere of vegetables, banana and coconut by random sampling. Entomopathogenic nematodes (EPN) were isolated from soil using *Corcyra cephalonica* larvae by insect trap method. EPN from insect cadavers were extracted by white trap method. From forty soil samples collected, three species of EPN were isolated and their morphological characters were studied.

The infectivity of native EPN strains were assessed at an inoculum level of 300 IJs/insect against test insects *viz.* termites, aphids, tobacco caterpillar and pseudostem weevil under *in vitro* condition. Among the isolates, isolate obtained from Mylom area in Kottarakkara (Isolate 2) showed maximum emergence of Infective Juveniles (IJs) from termites ($2.0x10^3$ IJs/adult), aphids ($1.2x10^3$ IJs/adult), tobacco caterpillar ($3.5x10^5$ IJs/larvae) and pseudostem weevil ($3.5x10^5$ IJs/grub). Isolate 2 also showed mortality percentage of 87.99, 86.00, 29.99 and 9.99 against termite, aphids, tobacco caterpillar and pseudostem weevil respectively at 24 hours after treatment. Isolate obtained from College of Agriculture, Vellayani (Isolate 1) and Isolate obtained from Kainidi area in Alappuzha (Isolate 3) showed mortality of 86.00 and 76.00 per cent in termites and 81.74 and 71.99 per cent in aphids respectively. Infection of Isolate 2 resulted in brown, pink and reddish brown discolouration in cadavers of termites, tobacco caterpillar and pseudostem weevil respectively.

Based on results of preliminary screening, Isolate 1, 2 and 3 were tested for their pathogenicity against test insects under *in vitro* condition. The experimental design used was CRD with treatments *viz.*, 10, 50, 100, 200 IJs, sterile water and chemical check and were replicated four times. Isolate 1 and 2 @ 100 IJs caused cent per cent mortality of termites at 48 hours after treatment (HAT) and it was statistically on par with chlorpyriphos 25 EC. Isolate 3 @ 200 IJs showed cent percent mortality of termites at 60 HAT. Isolate 2 @ 200IJs recorded 99.26 per cent mortality of aphids at 36 HAT and it was statistically on par with chemical, dimethoate 30 EC. Isolate 2 @ 100 IJs recorded cent percent mortality of aphids at 48 HAT. In the case of tobacco caterpillar, Isolate 2 @ 200 IJs recorded 65.08 and 80.52 per cent mortality at 48 and 60 HAT respectively. Isolate 2 @ 100 IJs recorded 92.53 per cent mortality of tobacco caterpillar at 72 HAT and it was statistically on par with chemical, flubendiamide 39.35 EC. Isolate 2 @ 200 IJs showed 62.66 per cent mortality of pseudostem weevil grubs at 72 HAT. Among the three isolates, Isolate 2 is proved to be the most potent EPN strain with highest mortality in termites, aphids, tobacco caterpillar and pseudostem weevil.

Based on the morphological characters, Isolate 1 was identified as *Steinernema* sp., Isolates 2 as *Metarhabditis* sp. and Isolate 3 as *Rhabditis* sp. The IJs of *Steinernema* sp. were specific in retaining the second stage cuticle. The adults were characterized by short stoma, excretory pore anterior to the nerve ring and a muscular oesophagous (female-154.00±5.57µm, male-144.40±4.14µm) without a well-defined butterfly valve in the basal bulb. The males had a C shaped body having pointed and curved spicules (44.10±4.46µm) with a boat shaped gubernaculum (23.70±2.98µm). The females had a sub median protruding vulva. The IJs of *Rhabditis* sp. had a narrow body without second stage cuticle. The adults of *Rhabditis* sp. had six unfused labium at the anterior region, long tubular stoma, excretory pore anterior to the basal bulb and a muscular oesophagous with a well-defined butterfly valve. *Metarhabditis* sp. (Isolate 2) had lobed oesophageal glands different from

Rhabditis sp. (Isolate 3). The males of *Metarhabditis* sp. (Isolate 2) had a round manubrium in spicules (39.63±3.59µm) unlike males of *Rhabditis* sp. (Isolate 3) (33.83±4.36µm). *Metarhabditis* sp. (Isolate 2) was characterized by presence of peloderan bursa with eight papillae whereas *Rhabditis* sp. (Isolate 3) had leptoderan bursa with nine papillae. The male tail in *Metarhabditis* sp. (Isolate 2) (28.60±5.10µm) was straight and pointed whereas *Rhabditis* sp. (Isolate 3) had a ventrally curved tail (29.71±4.86µm). Vulva was characterized by slightly protruding vulval lips in females of *Rhabditis* sp.

Based on the pathogenicity studies, Isolate 2 (*Metarhabditis* sp.) was identified as the most potent strain causing highest mortality in test insects. Hence molecular characterization of Isolate 2 was done and result revealed it as *Metarhabditis rainai.* This was the first report of *M. rainai* in India.

From the above study it could be concluded that *M. rainai* can be exploited as a successful biocontrol agent pertaining to its pathogenicity against termites, lepidopteran and coleopteran pests and effort needs to be directed towards formulating the strain to improve its efficiency and shelf life.