ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF CASHEW STEM AND ROOT BORER, *Plocaederus ferrugineus* L. (COLEOPTERA: CERAMBYCIDAE)

by

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THESIS

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DECLARATION

I, **DEEPAK S. POOJARY** hereby declare that this thesis entitled **"Entomopathogenic nematodes for the management of cashew stem and root borer, *Plocaederus ferrugineus* L. (Coleoptera: Cerambycidae)"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Entomopathogenic nematodes for the management of cashew stem and root borer, *Plocaederus ferrugineus* L. (Coleoptera: Cerambycidae)"** is a bonafide record of research work done independently by **Mr. Deepak S. Poojary** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Deepak S, Poojary

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LIST OF ABBREVIATIONS

cm	: Centimeter
mm	: Millimeter
m	: Meter
g	: Gram
kg	: Kilogram
MT	: Metric tonnes
ha	: hectare
CSRB	: Cashew stem and root borer
EPN	: Entomopathogenic nematode
Spp.	: Species
ml	: milliliter
IJs	: infective juveniles
˚C	: Degree celsius
BOD	: Biological oxygen demand
PTS	: Prothoracic shield
CRD	: Completely randomised design
RBD	: Randomised block design
UV	: Ultra violet
LT_{50}	: Median lethal time
WDG	: Water dispersible granules
h	: hours
DAI/HAI	: Days/hours after inoculation
w/w	: Weight by weight

Introduction

1. INTRODUCTION

Cashew, (*Anacardium occidentale* L.) belonging to the family Anacardiaceae, is one of the most important foreign exchange earning plantation crops of the country. India occupies first place in terms of area under cashew (9.5 lakh ha) and second place in production (6.53 lakh MT) as well as productivity (720 kg ha^{-1}) (Rupa and Adiga, 2011).

Cashew is infested by a number of insect pests, out of which cashew stem and root borer, *Plocaederus ferrugineus* (Linnaeus) (Coleoptera: Cerambycidae) is the most important pest of cashew in south India. This pest was first reported by Ayyar (1932). Cashew plantations of more than 15 years old are often seen infested with this pest.

The adult of cashew stem and root borer is a medium sized dark brown beetle which lays eggs under the loose bark on the tree trunk of cashew. The newly emerged grubs bore into the bark and feed on the soft tissues making tunnels in all directions. The openings of tunnels are seen plugged with a reddish mass of chewed fiber and excreta. A full grown, larva measures 7.5 cm in length and tunnels its way to the root region, where it forms calcareous shell for pupation. The duration of life cycle is almost one year and there are several overlapping generations in a year. *P. ferrugineus* damages the cambial tissues and hence the flow of sap is arrested. The tree is weakened and if the infestation continues, it will lead to the death of the tree.

Management measures against the borer include prophylactic treatments by swabbing the tree trunk region and exposed roots with a suspension of mud slurry + carbaryl or coal tar and kerosene (1:2) or 5 per cent neem oil at regular intervals. Curative measures such as removal of grubs can be effective only in early stages of infestation.

The increasing awareness about the environmental and health hazards caused by chemical insecticides and the demand for organically grown

agricultural products in the international trade have drawn the attention of plant protection scientists towards naturally occurring bioagents in general and entomopathogenic nematodes (EPN) in particular.

Nematodes are microscopic, non-segmented, elongated roundworms that are colourless and lack appendages. Most nematodes are saprophytic in nature but a few are plant parasitic and some parasitize animals including insects. The latter group called as entomopathogenic nematodes are widely used for biocontrol of insect pests especially soil borne pests.

Entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae have very high biological control potential and have been used worldwide to manage insect pests of several crops (Kaya and Gaugler, 1993; Gaugler, 2002). With the realization of the harmful effects of the agrochemicals and the increasing thrust on the ecologically viable alternatives for insect pest management, entomopathogenic nematodes have emerged as a substitute for chemical insecticides.

The entomopathogenic nematodes have been utilized as very effective biocontrol agents against a wide range of insect pests. Their foraging nature, quick knock down effect and ability to survive for long periods in soil makes them excellent candidates for biocontrol of insect pests. In this background, the present study entitled "Entomopathogenic nematodes for the management of cashew stem and root borer, *Plocaederus ferrugineus* L. (Coleoptera: Cerambycidae)'' has been undertaken to evaluate the potential of different species of entomopathogenic nematodes as biocontrol agents of cashew stem and root borer.

The following are the objectives of the study:

1) To identify the potent species of entomopathogenic nematodes (EPN) against cashew stem and root borer.
2) To fix the optimum level of inoculum of EPN required to cause mortality in cashew stem and root borer.
3) To analyze the foraging behaviour of EPN species.
4) To test the field efficacy of EPN species against cashew stem and root borer.

Review of Literature

2. REVIEW OF LITERATURE

Cashew (*Anacardium occidentale* L.) is a very important foreign exchange earning crop of India. It was originally introduced into India from Brazil by Portuguese travellers during sixteenth century. Initially, it was considered as a suitable crop for soil conservation, afforestation and also wasteland development, but gradually gained commercial importance. Cashew is now widely grown in tropical climates and it has very well adapted to the Indian conditions. Now India is the largest producer, processor, consumer and exporter of cashew in the world. In India, it is mainly cultivated in Maharashtra, Goa, Karnataka and Kerala along the West coast and Tamil Nadu, Pondicherry, Andhra Pradesh, Orissa and West Bengal along the East coast. At present Maharashtra is ranking first in area, production and productivity in the country.

Pest infestation is a major constraint in cashew production. It is attacked by a number of insect pests during different stages of its growth and development. More than fifty species of insects are known to be infesting cashew in India in different degrees of intensity. However, when the extent of damage is taken into account only four are considered to be major pests which includes stem and root borer, tea mosquito bug, leaf miner, apple and nut borer. The other minor pest includes thrips, leaf and bloosm webber and mealy bug (Maruthadurai *et al*., 2012).

The cashew stem and root borer (CSRB), *Plocaederus ferrugineus* is a major pests of cashew in all cashew growing tracts of India (Abraham, 1958, Pillai, 1976) and a few other cashew growing countries (Asogwa *et.al.,* 2009) and pose a serious problem in realizing the maximum yield potential of cashew nut. The grubs form irregular tunnels in the cashew bark of the stem and roots, thereby damage vascular tissues resulting in gradual yellowing of the foliage of yielding trees and subsequently lead to death of trees, thereby reducing the tree population. The presence of grubs can be recognized by the exudation of frass and gum in the infested region, usually in the collar zone in early stages and gum as well as

coarse frass, yellowing canopy at the later stage of infestation (Vasanthi and Raviprasad, 2013).

The biology and morphometric study indicated that, mean grub period was 163.10 ± 20.55 and 168.37± 30.32 days, mean pupal period was 144.33 ± 29.57 and 145.16 ± 29.10 days for developing as males and females, respectively. The total development period ranged between 261-363 days for male and 242-356 days for female. The morphometric study indicated that the mean body length was 38.8 mm; mean body width being 8.76 mm and mean body weight being 0.89 g; the females had mean body length of 33.5 mm; mean body width of 10.29 mm and mean body weight of 1.39 g (Vasanthi and Raviprasad, 2013)

Management measures against *P. ferrugineus* includes prophylactic treatments by swabbing the trunk with a mixture of coal tar and kerosene at the ratio of 1:2 or chloropyriphos 0.02 per cent twice a year once during March-April and again during November-December. Root feeding with monocrotophos at 20 ml/tree was also promising. Curative measures such as removal of grubs can be effective only in early stages of infestation.

2.1. BIOLOGICAL CONTROL

The use of chemical pesticides such as insecticides, herbicides, fungicides and acaricides have proved to be harmful to the environment and non target organisms, so that biological control methods have greater importance now a days. In addition to natural agents, there is a concept of biological plant protection which is the use of biological processes to improve plant growth and modify plants to be resistant against disease and also develop effective ways to suppress pests (Georgis *et al.*, 2006). The main objective of pest management projects is to ensure that pest populations are reduced and crop damage is also directly reduced so that people can purchase good quality fruits, vegetables and other plant products (Flint and Dreistadt, 1998).

The main aim of biological control is to use natural enemies to destroy or reduce pests by disrupting their reproduction and then ultimately decrease pest population (Flint *et al.,* 1998). Natural enemies play an important role in pest management and can be used to specifically target unwanted host in an ecosystem. These enemies can be parasites or predators both with the mission to attack and kill the pests. Bacteria, fungi and nematodes can be used as natural enemies. Several nematodes species have been identified to have the potential to successfully kill specific insect host within a few days after invasion, and examples include *Heterorhibditis* and *Steinernema* species (Jagdale *et al.*, 2009).

Very often natural enemies are not noticed by pest managers and are left unidentified, even though it is a fact that every pest has its own natural enemy that can decrease its population when conditions are favourable. Usually natural enemies are destroyed by the application of synthetic chemical pesticides and also unfavourable weather conditions which also contribute to them not being noticed by pest managers.

The problem with synthetic chemical pesticides has been reported based on animal and human health implications, as well as the ecological problems caused by application of these synthetic chemicals. The accumulation of these chemicals in human can interfere with most of the biological processes and pathways; affecting several proteins involved in cell growth and repair because they can induce DNA mutations in human cells (Segal and Glazer, 2000). These pesticides further affect the ecosystem in nature whereby they kill the beneficial insects as most of them are not host specific. Another disadvantage is associated with the resistance of the insects towards the synthetic chemical pesticides whereby even at the highest concentration of the chemical, the insects rapidly grow and continue damaging crops (Georgis *et al.,* 2006). A better way of controlling and reducing pests is to find suitable alternative. In this context the use of biological agents has so far not shown any adverse consequences towards human health and therefore considered as safe for human health as well as the environment. The identification of the naturally occurring enemies is important in taking optimum advantage of

the use of biological control (Flint and Dreistadt, 1998) and this is why this research aims to identify entomopathogenic nematodes which will be tested for their ability to be used for biological control of cashew stem and root borer, *P. ferrugineus*.

2.2. ENTOMOPATHOGENIC NEMATODES

Entomopathogenic nematodes (EPNs) are non-segmented roundworms also known as threadworms or eelworms. They have an excretory, nervous, digestive and reproductive system but lack the circulatory and respiratory system. The alimentary tract of EPNs consists of the mouth, buccal cavity or stoma, oesophagus, intestine, rectum and the anus respectively. The size ofEPN ranging from 0.3 to 10mm long and they can be more or less cylindrical (Flint and Dreistadt, 1998).

EPNs are found in various locations and can survive externally on insect exoskeleton or internally in the reproductive, respiratory, digestive or excretory system. Generally some nematodes reside in very dry areas including deserts and most have the ability to tolerate environmental stresses which include anoxybiosis, thermobiosis and desiccation (Grewal, 2000).

2.2.1. History of EPN

Entomopathogenic nematodes (EPN) have been known since 1923 when Steiner described the first species *Aplectana kraussei* Steiner, collected by Krausse in Germany from a hymenopterous sawfly (Krausse, 1917). The species was later renamed *S. kraussei* by Travassos (Travassos, 1927). Six years later Steiner described another species *Neoaplectana glaseri* (Steiner, 1929) reported by Glaser and Fox (1930) from Japanese beetle larvae *Popillia japonica* in New Jersey, USA. For over 50 years these two nematodes were regarded as two separate genera, but in 1982, Wouts, a New Zealand Nematologist proved that *Neoaplectana* Steiner to be the junior synonym of *Steinernema* Travassos (Wouts *et al.*, 1982). After the description of the new species *S. feltiae*, *Steinernema* was

placed in the family Steinernematidae (Filipjev, 1934) in the order Rhabditida. The second family and genus of entomopathogenic nematodes were described by Poinar (1976) as Heterorhabditidae and *Heterorhabditis* respectively. The type species in this family was *Heterorhabditis bacteriophora* Poinar, 1976. In the family Steinernematidae, a second genus, *Neosteinernema* with a single species, *N. longicurvicauda*, was described by Nguyen and Smart (1994), which isolated from the termite, *Reticulitermes flavipes* Kollar.

For nematodes to be entomopathogenic, they must search, penetrate, release the symbiotic bacteria, kill and reproduce within the insect cadaver and then emerge as dauer juveniles (Kaya and Gaugler, 1993). Recently, *Oschieus carolinensis* and associated bacterium *Serratia marcessens* (Torres-Barragan *et al.*, 2011) and *O. chongmingensis* (formerly *Heterorhabditidoides chongmingensis*) with bacterium *S. nematophila* (Zhang *et al.*, 2008) have also shown pathogenicity to insects and are defined as entomopathogenic nematodes. The nematode *Caenorhabditis briggsae* (Abebe *et al.*, 2010) has insect pathogenic bacteria *Serratia*, (Abebe *et al.*, 2011) but is less virulent to the insect host, and is not considered as entomopathogenic nematode (Dillman *et al.*, 2012).

The most studied and commercially available entomopathogenic nematodes (order: Rhabditida) belong to two families: Steinernematidae and Heterorhabditidae. These soil-dwelling nematodes are obligate and lethal parasites of insects (Poinar, 1990). They can provide effective biological control of some important lepidopteran (Glazer and Navon, 1990; Gouge *et al.*, 1999), dipteran (Scheepmaker *et al.*, 1998; Willmott *et al.*, 2002; Nielsen, 2003), homopteran (English-Loeb *et al.*, 1999; Cuthbertson *et al.*, 2003), and coleopteran (Journey and Ostlie, 2000; Shapiro-Ilan *et al.*, 2003; Ansari *et al.*, 2008; Ellis *et al.*, 2010), orthopteran and isopteran (Ganguly and Gavas 2004a) pests of commercial crops and are amenable to large-scale culture in artificial media (Friedman, 1990; Ehlers, 2001; Gaugler and Han, 2002; Shapiro-Ilan and Gaugler, 2002). Apart from insects, a terrestrial pillbug (crustacean) has shown susceptibility to EPNs (Ganguly and Gavas 2004a).

Entomopathogenic nematodes are unique among Rhabditids in having a symbiotic relationship with an enteric bacterium species (Thomas and Poinar, 1979; Brehelin *et al.*, 1993). The bacterial symbiont is required to kill the insect host and to digest the host tissues, thereby providing suitable nutrient conditions for nematode growth and development inside the body of the host (Boemare, 2002).

2.2.2. Survival of EPN

EPNs can survive in wide range of climate. The temperature, moisture and pH requirement of EPNs varies with the species. Sunanda *et al.* (2012) studied the effect of temperature on longevity of *S. abbasi* and *H. indica* under laboratory conditions and reported that survival of both the nematodes was highest at 30^0 C when the exposure period was 15 days. Survival of both the EPNs gradually declined with an increase in time period and also declined at < 30^0 C and > 40^0 C.

Gulsarbanu and Rajendran (2003) tested effect of UV radiation on survival and infectivity of three entomopathogenic nematodes and reported that absolute mortality was recorded with continuous exposure to UV radiation for 120 – 135 min. The median lethal time (LT_{50}) for *H. indica*, native *Steinernema sp*. and *S. glaseri* was 33.51, 45.88 and 38.45 min/ larva respectively indicating that native isolate of *Steinernema* sp. was more tolerant to UV light than the other two nematodes.

Hussaini *et al.* (2004) conducted study on effect of pH on survival of some indigenous isolates of entomopathogenic nematodes, *S. carpocapsae* PDBC EN 11 and *H. indica* PDBC EN 13.3 and reported that maximum survival for *H. indica* was obtained at pH 5 and for *S. carpocapsae* at 7 and least at pH 2 and 3, respectively.

Ganguly and Singh (2001) conducted an experiment on temperature requirement for infectivity and development of an indigenous EPN, *S. thermophilum* and observed 100 per cent larval mortality at 35^0 C within 36 h

after inoculation, while at 25 and 30^0 C, it occurred within 48 HAI and at 20^0 C within 216 HAI.

Shyam Prasad and Singh (2003) conducted a study on requirement of soil temperature, soil moisture and relative humidity for the survival of EPN and found that for the survival and pathogenicity of *Heterorhabditis* spp., 8 per cent of soil moisture and temperature of 24^0 C at relative humidity of 100 per cent were optimum to cause the mean pathogenicity of 75.27 per cent.

2.2.3. Biology and lifecycle of EPN

Entomopathogenic nematodes cause mortality of insects in a three-step process. Firstly, the nematode has to migrate, searching for the target host. Secondly, upon contact with the host, it has to penetrate through natural body openings, such as anus, mouth, spiracles, and through the cuticle. Finally, the nematode-bacteria complex should be able to overcome the insect's immune system and to multiply for producing new generations of infective juveniles.

EPN have a life cycle which includes the egg stage, four juvenile stages and the adult stage. Both *Steinernema* and *Heterorhabditis* species have a free-living third stage juvenile (J3), termed a dauer juvenile, that is the infective stage and therefore also called as infective juvenile (IJ). These IJs harbour a symbiotic bacterium (Endo and Nickle, 1995), which plays an essential role in subsequent stages of the life cycle. Bacterial symbionts are found in a modified ventricular part of the intestine in *Steinernema*, as well as throughout the intestinal lumen in *Heterorhabditis* (Hominick, 1990). The IJs seek out a suitable host and instigate the infection process.

The IJs of *Steinernema* enter the insect body through natural openings such as the mouth, anus and spiracles. Infective juveniles of *Heterorhabditis*, however, enter through natural openings and the body wall. These nematodes possess a dorsal tooth in the anterior region of head which helps them to gain entry into the haemocoel by breaking the thin cuticle of intersegmental membrane (Bedding and

Molyneux, 1982; Cui *et al.*, 1993). In next step, infective juveniles release cells of symbiotic bacteria from its intestine into the haemocoel of the insect (Akhurst, 1982). These bacterial cells are released by regurgitation (Ciche and Ensign, 2003) through the mouth during infection of the insect larva. The IJs recover or exit from the developmentally-arrested third, non-feeding stage, triggered by either bacterial or insect food signals (Strauch and Ehlers, 1998).

The insect haemolymph provides a rich medium for the bacterial cells and these begin to grow, release toxins and exoenzymes and kill the insect within two days (Han and Ehlers, 2000). These bacteria also produce antibiotics and other noxious substances that protect the host cadaver from other microbes in the soil (Webster *et al.*, 2002). Nematodes resume development, start feeding on bacteria and moult to the fourth stage and reach adulthood within 2-3 days. Nematode development continues over two to three generations until the nutrient status of the cadaver deteriorates whereupon adult development is suppressed and IJs accumulate. These non-feeding infective stages emerge into the soil where they may survive for several months in the absence of a suitable host (Hominick, 1990).

Steinernematids exhibit amphimictic reproduction (Poinar, 1990). The IJ of *Steinernema* matures to become either a male or female (Dix *et al.*, 1994). After mating, females lay eggs that hatch as first stage juveniles that moult successively to second, third and fourth stage juveniles and to males and females of the second generation. Depending upon the food availablity, up to three generations can develop inside the dead host (Wang and Bedding, 1996). Upon depletion of the food resources or overcrowding inside the host cadaver, the formation of developmentally-arrested third stage infective juveniles occur which leave the cadaver in search of new host (San-Blas *et al.*, 2008).

In *Heterorhabditis* by contrast, the IJs mature to give first generation hermaphrodite females, but these females give rise to a second generation of amphimictic males and females and to self-fertile hermaphroditic females and IJs

(Dix *et al.*, 1992; Strauch *et al.*, 1994). The development into amphimictic adults is induced by favourable nutritional conditions, whereas the development of hermaphrodites is induced by low concentrations of nutrients (Strauch *et al.*, 1994).

In both genera, *Steinernema* and *Heterorhabditis*, as long as abundant nutrients are available, additional adult generations develop. When the nutrients are consumed, the late second stage juveniles cease feeding, incorporate a pellet of symbiotic bacteria in their bacterial chamber (Popiel *et al.*, 1989) and moult to the third stage juvenile. They retain the cuticle of the second stage as a sheath, and leave the cadaver in search of new hosts. They may survive for several months in the absence of a suitable host. The cycle from entry of IJs into a host to emergence of new IJs is temperature dependent and varies for different species and strains. It generally takes about 6-18 days at temperatures ranging from 18 to 28 °C in *G. mellonella* (Poinar, 1990; Nguyen and Smart, 1992).

2.2.4. Nematode- Bacterial symbiosis

The Steinernematids have symbiotic association with bacteria in the genus *Xenorhabdus* (Thomas and Poinar, 1979) whilst the *Heterorhabditis* have a bioluminescent symbiont in the genus *Photorhabdus* (Boemare *et al.*, 1993). The relationship between the parasitic nematodes and bacteria is highly specific (Akhurst and Boemare, 1990; Bonifassi *et al.*, 1999). Symbiotic bacteria of both genera are motile, gram-negative and belong to the family Enterobacteriaceae. The relationship of *Xenorhabdus* with *Steinernema* and that of *Photorhabdus* with *Heterorhabditis* is reported to be obligatory (Boemare *et al.*, 1997; Forst *et al.*, 1997). Each nematode species is primarily associated with a single bacterial species (Akhurst and Boemare, 1990). In the case of *Steinernema,* the *Xenorhabdus* symbionts are present in the intestinal vesicle (Bird and Akhurst, 1983), while in *Heterorhabditis*, *Photorhabdus* cells stick together in the anterior part of infective juvenile guts (Boemare *et al.*, 1996). In the course of isolating bacteria from infective juveniles of the entomopathogenic nematode *Steinernema*

thermophilum Ganguly and Singh, 2000, a novel species of bacteria, *Providencia vermicola* was identified (Somvanshi *et al.,* 2006).

The bacteria provide nutrients to the nematodes, produce antibiotics that inhibit competing microbes, and kill the host through septicaemia (Akhurst, 1982; Akhurst and Boemare, 1990). At the end of the bacterial multiplication, production of a large variety of antimicrobial compounds prevents microbial contamination, mainly from the insect intestinal microflora (Akhurst, 1980; Akhurst, 1982; Webster *et al.*, 1998). Thus, bacteria create suitable conditions for the development of their nematode host in the insect cadaver. Although the nematodes may also contribute to host death through suppression of the immune system and toxin production (Akhurst and Boemare, 1990; Sim and Rosa, 1996).

The most important role they play in mutualism is serving as vectors for the bacteria. These symbiotic bacteria cannot survive in the natural environment and are generally not pathogenic when ingested by a host (Akhurst and Boemare, 1990; Morgan *et al.*, 1997). Therefore the perennial symbiosis is maintained by ingestion of some bacterial cells by the nematode dauer juveniles before leaving the insect (Boemare, 2002).

The cyclic association between the symbiotic bacteria and EPN starts and ends with the IJs in the soil. The life cycle can be divided into three stages. They are release of symbiotic bacteria into the insect haemolymph, the bacterial cells begin to grow and death of the insect follows, through septicaemia (Boemare *et al.*, 1997; Forst *et al.*, 1997). Some strains of *Xenorhabdus* and *Photorhabdus* are highly virulent, injection of fewer than ten cells of the bacterium into the haemocoel may be sufficient to kill a susceptible insect such as *G. mellonella* (Poinar and Thomas, 1967; Forst *et al.*, 1997;).

Ansari *et al*. (2003) reported 100 per cent mortality of *Heterorhabditis philanthus* third instar larvae 72 h after injection with 25 viable cells of *P. luminescens* and *X. bovienii*. As the bacteria enter the stationary phase of their

growth cycle they secrete lipase, protease and several broad spectrum antibacterial and antifungal antibiotics (Akhurst and Boemare, 1990; Forst and Nealson, 1996). The likely role for the degradative enzymes is to break down the insect tissues thereby providing a rich food supply for the developing nematode. Nematode reproduction is optimal when the natural symbionts dominate the microbial flora, suggesting that the bacteria can serve as a food source and/or provide essential nutrients that are required for efficient nematode proliferation (Akhurst and Boemare, 1990; Poinar, 1990). Symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*) of EPN occur in two phenotypic forms: phase I and phase II. Phase I cells are larger than phase II cells and produce significantly greater amounts of exoenzymes, toxins and antibiotics than phase II forms. However, the IJs package and transport only phase I cells. These bacterial symbionts are not isolated from soil and it is generally assumed that they cannot exist in the soil environment in the absence of their nematode associates (Burnell and Stock, 2000).

2.2.5. Mass production, Formulation and Storage of EPN

Entomopathogenic nematodes can be mass produced *in vivo* and *in vitro* (Friedman, 1990; Shapiro-Ilan and Gaugler, 2002). The most commonly used insect host for *in vivo* production is the greater wax moth, *Galleria mellonella* larvae (Lindegren *et al.*, 1993; Flanders *et al.*, 1996; Kaya and Stock, 1997) because of its high susceptibility, commercial availability as a food for birds, lizards and fish baits, and high yield (Woodring and Kaya, 1988). While *in vitro* mass production is achieved via a solid medium (Bedding, 1981 and 1984) or in liquid culture (Friedman, 1990; Ehlers, 2001), *in vitro* mass production has advantage in terms of economy of scale over *in vivo* production. However, *in vivo* culturing is still useful for research and can also be used in developing countries as a cottage industry for commercial application (Gaugler and Han, 2002).

The first attempt at *in vitro* mass production was not very successful due to a lack of knowledge about the symbiotic bacteria (Glaser, 1932). However,

much later after the discovery of bacterial symbionts their importance realized for the reproduction of *S. carpocapsae*, a foundation was laid down for the *in vitro* mass production of entomopathogenic nematodes (Poinar and Thomas, 1966). The EPN were grown on artificial medium consisting of nutrient broth, yeast extract and vegetable oil, cooked with flour and coated onto polyether polyurethane sponge and incubated for 3 days on 25 °C in a flask. A yield of up to 10 million was obtained in one month (Wouts, 1981). However, Bedding developed a monoxenic solid culture, which was successfully commercialized for the first time (Bedding, 1981 and 1984). In this method, the author used the same ingredients as that of Wouts (1981) but fortified the medium additionally with emulsified beef-fat and pig's kidneys generating a higher yield of infective juveniles/ g medium (Bedding, 1984).

Friedman (1990) reported the development of technique for large-scale production in liquid culture using 80,000 litre fermenters and achieved a yield of $50x10^{12}$ IJs/month. His method decreased the cost of production as well. The first liquid culture production and commercialisation of EPN was initiated by the company Biosys in 1992 (Ehlers, 1996). Nowadays most of the EPN are produced by companies situated in Europe and the United States using liquid culture fermentation. Recent improvements in liquid culture fermentation has not only increased the yield but also has commercialised more nematode species, such as: *S. carpocapsae*, *S. feltiae*, *S. glaseri*, *S. scapterisci*, *S. riobravis*, *S. kraussei*, *H. bacteriophora*, *H. indica*, *H. megidis*, and *H. marelatus* (Kaya *et al.*, 2006).

EPN are available in different formulations (Grewal, 2002) which was the next step following mass production. While formulating nematodes, two things are deemed necessary, a long shelf-life of nematodes and their ease in application. The simplest method is impregnating a moist substrate (such as sponge, cedar shavings, peat, vermiculite, etc.) with nematodes. The sponge needs to be squeezed in water before application, to release the nematodes, whereas nematodes from other carriers could be applied directly to the soil as mulch. However, these formulations are labour-intensive, require continuous refrigeration

and lack economy of scale and therefore can be used on small scale only. Some of the better formulations developed include activated charcoal (Yukawa and Pitt, 1985), calcium alginate (Kaya and Nelsen, 1985; Georgis, 1990; Navon *et al.*, 1998), flowable gels (Georgis and Manweiler, 1994), wheat flour (Connick *et al.*, 1993) and attapalgite clay chips (Bedding, 1988).

Though calcium alginate formulation increases the shelf-life of *S. carpocapsae* up to 3-4 months, commercially it was not viable. One of the better formulations like Water Dispersible Granules (WDG) gave maximum shelf-life of up to 6 months for the comparatively storage-stable nematode, *S. carpocapsae* (Silver *et al.*, 1995). The improvement in shelf-life was attributed to the induction of partial anhydrobiosis by a mixture of ingredients in the formulation such as silica, clays, cellulose, lignin, and starches (Grewal, 2000). Nematode-infected cadavers have also been used (Shapiro-Ilan *et al.*, 2001; Shapiro-Ilan *et al.*, 2003a; Ansari *et al.*, 2009), but these formulations lack economy of scale since they involve *in vivo* production and there are difficulties in dose determination, storage and application (Gaugler and Han, 2002)

Entomopathogenic nematodes can be applied easily with conventional spray equipments, and are exempt from registration in many countries (Kaya and Gaugler, 1993; Lacey *et al.*, 2001). However, despite of all these advantages, the limited shelf-life places a major barrier to their long-term commercial application. The shelf-life of partially-desiccated infective juveniles in the water dispersible granules (WDG) formulation at 25°C around 5-6 months for *S. carpocapsae*, two months for *S. feltiae*, and one month for *S. riobrave* (Grewal, 2000). This formulation however, is no more available.

The limited shelf-life of EPN has been addressed by many authors (Selvan *et al.*, 1993; Surrey and Wharton, 1995; Grewal, 2000; Grewal and Jagdale, 2002; Ehlers *et al.*, 2005). These studies have focused on increasing the longevity and storage of EPN through improved formulations and the induction of partial desiccation and potentially anhydrobiosis. Anhydrobiosis slows down metabolic

activity and enables the nematode to preserve their fat stores, allowing them to live for long (Grewal, 2000). However, a long-term storage method based on desiccation has not met with complete success (Womersley, 1990). Nematode cryopreservation allows EPN to be stored for experimental purposes but not in commercial quantities and at high cost (Popiel and Vasquez, 1991; Curran *et al.*, 1992). This necessitates the search for alternative methods for the long-term storage of entomopathogenic nematodes.

Nema Gel, a novel formulation for entomopathogenic nematodes, based on a newly developed hydrogel, has been found to enhance the shelf life of the indigenous nematode *S. thermophilum*. The nematode concentration in the Nema Gel can be adjusted up to 1×10^5 infective juveniles per gram. The survival of formulated nematodes was significantly better than that in aqueous suspensions after 9 months storage at 15 °C. More than 50 per cent survival of formulated infective juveniles was recorded even after 36 months storage at 15 °C, and 24, 16 and 8 weeks of storage at 30, 35 and 40 °C, respectively. Formulated nematodes stored at room temperature (exposed to fluctuating diurnal temperature conditions varying from 15 to 39 °C), showed 89 per cent survival compared to only 16 per cent in control, after 9 months of storage (Ganguly *et al.,* 2008).

2.3 LABORATORY STUDY ON PATHOGENECITY OF EPN

The potential of entomopathogenic nematodes for insect control has been fully realized and well documented, and the number of researchers studying them has increased considerably during the last few decades. This novel area of research has drawn the attention of Entomologists and Nematologists. Several new strains have been isolated and tested for their biocontrol potential against the insect pest of crops. There has been exponential growth in the description of new species, as for example there were only 9 species of *Steinernema* in 1990, which has now grown to 56 (Ganguly, 2006).

Stark and Lacey (1999) evaluated the susceptibility of western cherry fruit fly (Diptera;Tephritidae) to five species of entomopathogenic nematodes under laboratory conditions. Infestations by *S. carpocapsae* and *S. feltiae* caused significantly higher pupal mortality than *S. riobrave*. Eventhough *H. bacteriophora* killed greater percentage of pupae than *S. feltiae*, the reproductive potentials of two species within the puparia were fairly equal. Arthurs *et al*. (2004) studied the efficacy of *S. carpocapse* and reported that infection rate was 34 per cent higher among borers (representing a 65 per cent increase) compared with exposed foliar pests.

Madhu (1989) studied the efficacy of entomopathogenic nematode DD-136 (*Neoplectana carpocapse* Weiser) on CSRB and reported that the nematode induced maximum mortality in first instar grubs followed by second instar grubs. However, the nematodes failed to control third, fourth and fifth instar grubs at the dose of 135 – 380 IJs per third instars of CSRB grubs. On contrary, Gavas (2012) reported that exposure to higher concentration of entomopathogenic nematode, *H. bacteriophora* resulted in cent per cent mortality of CSRB grubs within 48-72 hours.

Although, several species of Steinernema already known from different parts of the world, yet the search for indigenous species and strains is extremely essential which can establish easily in Indian agroclimatic condition. *S. thermophilum* (IARI- EPN 1) has been described from India by Ganguly and Singh, 2000 which is the first new species of this genus from this country.

The host range study showed that the IJs of *S. thermophilum* infected and reproduced on many different orders of insects (Gavas, 2001, Ganguly and Gavas 2004a). These included seven species in Lepidoptera, four in Orthoptera, and one each in Hemiptera, Isoptera, and Coleoptera. The higher nematode inoculum level (500 IJs/insect) resulted in higher insect mortality and nematode development compared with the lower inoculum level (50 IJs/insect). A terrestrial pillbug (crustacean) was also susceptible to the nematode, but two pollinating

hymenopteran insects (rock bee and Indian bee) and two blattodean species (American and German cockroach) were not susceptible to infection at the tested concentration.

S. thermophilum infected greater wax moth larvae at a temperature range from 10 to 35 °C and reproduced from 20 to 35 °C. It can access the host at all the moisture level tested, varying from 1 to 19 per cent (w/w). Insect mortality increased significantly at 3 per cent moisture and mortality gradually increased up to the 9 per cent moisture level, and thereafter, it declined. Based on these results, the optimum temperature range for infection and reproduction of *S. thermophilum* was 25–35 °C, and the optimum soil moisture level was 9 per cent with optimal range of 3 to 16 per cent (Gavas, 2001, Ganguly and Gavas 2004b).

Mannion and Jansson (1992) assessed the virulence of ten entomopathogenic nematodes to *Cylas formicarius*. Most nematodes were more virulent to larvae than to pupae. Adults were less susceptible to nematodes than other stages, and adult males were more susceptible than females.

Hypothenemus hampei is a major pest of coffee seeds in Brazil and other South American countries (Waterhouse, 1998). Infestations of *H. hampei* occur in coffee seeds when remaining in berries on the trees and in berries that fall to the ground. Spraying of nematodes on fallen berries might remove the need to collect them, leaving them to produce mulch. Dispersal of infected adults may also spread nematodes into the pest population. According to Waterhouse (1998) there appears to be only one record of nematodes attacking *H. hampei* in the field in India (Varaprasad *et al.,* 1994). Allard and Moore (1989) showed that a *Heterorhabditis sp.* could cause high mortality of both adult and larvae. Castillo and Marban (1996) reported differences in the infectivity of eight nematode strains to *H. hampei* larvae and found that three strains of *Heterorhabditis sp.* and one of *S. carpocapsae* caused high mortality of the larvae.

Cannayane *et al.* (2007) evaluated the pathogenicity of a pair of EPN species (*H. indica* and *S. glaseri*) against root grub, *Basilepta fulvicorne* in cardamom and reported that, mortality of grubs was brought about by both the EPNs within 48 h at the inoculums level of 100 IJs/ 0.5 ml of sterile water. The highest mortality of 95.3 per cent was observed with *H. indica* treatment in 96 h than with *S. glaseri*, which showed 78.6 per cent infection at the same time interval.

Farooq *et al*. (2000) successfully cultured *H. bacteriophora* and *S. carpocapsae* on third instar of silk worm larvae and reported that these two species could able to bring mortality in *Corcyra cephalonica* and *Agrotis ipsilon* within 96 h of treatment.

Padmanaban *et al*. (2002) conducted a experiment to determine the effect of entomopathogenic nematode, *H. indica* on banana stem weevil, *Odoiporus longicollis* and reported that, after 72 h of inoculation, there was mortality of insects to the tune of 33.3 per cent in the treatment with 10- 70 IJs and 66.6 per cent in the treatment with 80-100 IJs per grub.

Kesavakumar and Ganguly (2011) conducted experiment on Indian strains of EPN against different Homopterans under laboratory conditions and reported that *S. thermophilum* caused significant mortality of mealy bug (*Phenacoccus solenopsis*), which was 83 per cent within 72 h after inoculation at 50 IJs/ ml and 100 per cent within 48 h at 500 IJs/ml. *S.riobrave* and *S. harryi* produced intermediate mortality of about 66 per cent within 60 h at 500 IJs/ ml. Against aphid *S. thermophilum* caused 66 and 83 per cent of mortality at 50 and 500 IJs/ml, respectively within 3 days post inoculation.

Nickdel *et al*. (2009) conducted experiment on penetration of *H.bacteriophora* and *S. bicornutum* on oak acorn weevil, *Curculio glandium* Marsham, under laboratory conditions using 4000 IJs/ml of sterile water and found that, penetration percentage was 1.6 for *H. bacteriophora* and 0.55 for

S. bicornutum. In another experiment, *H. bacteriophora* and *S. bicornutum* were applied at different concentrations (0, 150, 250, 500, 1000 and 2000 IJs per 1 ml of distilled water) in 9 cm Petri plates lined with filter papers in order to test their capability of parasitization of the fifth instar larvae of *C. glandium*. Maximum mortality caused by *H. bacteriophora* and *S. bicornutum* were 58.3, 25 per cent (at 21-24°C) and 63.5, 30.5 per cent (at 25-28°C).

Hussaini *et al.* (2002) found that median lethal concentrations (LC_{50}) of *S. bicornutum* (PDBC EN 3.2) and *H. indica* (PDBC EN 13.3) against *A. ipsilon* were 64 and 34 infective juveniles (IJs) per larva, respectively, at 72 h post inoculation by dose response assay. Time assay response with the same revealed LT_{50} values of 72 and 58 h, respectively.

Kowalska and Jakubowska (2007) studied susceptibility of *A. segetum* fifth-instar larvae to *Steinernema bicornutum* Sb360 and *S. feltiae* at 50 larvae/cm^2 under laboratory and semi-field conditions in Poland and observed larval mortality of 70 and 55 per cent in *S. feltiae* and *S. bicornutum*, respectively, at 50 larvae/cm^2. Under semi-field conditions, only 20 per cent mortality observed in *S. feltiae*.

Kary *et al.* (2010) conducted a study by using *H. bacteriophora, S. bicornutum, S. carpocapsae* and *S.feltiae* against colorado potato beetle, *Leptinotarsa decemlineata* at the concentration of 100, 200, 400, 500 and 1000 infective juvenile (IJs) per individual under laboratory conditions. Research proved that *H. bacteriophora* IRA10 had the highest toxicity and *S. bicornutum* IRA7 was the lowest one. There were no significant differences between strains at lowest concentration in all exposure times.

Nielsen and Philipsen (2005) conducted a lab study on susceptibility of pupating larvae of pollen beetles, *Meligethes* spp. Stephens (Coleoptera: Nitidulidae) to *Steinernema bicornutum* and reported that successful pupation of pollen beetle larvae was reduced with increasing number of nematodes.

Alves *et al.* (2009) studied the susceptibility of *Hedypathes betulinus* (Coleoptera: Cerambycidae) to the *S. carpocapsae* (Nematoda, Steinernematidae) in the laboratory. The nematode was applied in 4 doses (12.5; 25; 50 and 100 infective juveniles/cm^2), against adult insects. The nematode caused mortality of 35 and 76 per cent at the smallest and largest concentrations, respectively.

2.4. FORAGING BEHAVIOUR OF ENTOMOPATHOGENIC NEMATODES

The optimum soil depth at which various entomopathogenic nematode can access the insect host is primarily governed by the size of the infective juveniles, their foraging behaviour and ecological traits. Various studies have shown that *S. carpocapsae* nictates (Kondo and Ishibashi, 1986), tends to stay near the soil surface and does not disperse far (Moyle and Kaya, 1981) and it is adapted to infect mobile hosts on soil surface. Whereas *S. glaseri* and *H. bacteriophora* occurs deeper in the soil and travel much farther (Schroeder and Beavers, 1987).

Comparitive studies on the infectivity of *S. carpocapsae, S. cubanum, S. glaseri, S. monticolum and S. rarum* at different soil depths were conducted by some workers (Koppenhofer *et al.,* 1995; Koppenhofer and Kaya, 1998), which revealed that *S. carpocapsae, S. monticolum and S. rarum* could not infect at 10 cm depth. *S. cubanum* and *S. glaseri* exhibited maximum infectivity at 5 and 10 cm depths. Significant difference were evident in the establishment rates of infective juveniles among different soil depth within the species, and also between various species of nematodes. *S. carpocapsae* infect more insects on the surface, while cruising predators like *H. bacteriophora* infect insects that live deep in the soil (Campbell and Gaugler, 1993).

The foraging behaviour of *S. thermophilum* was conducted at various soil depth (0, 2, 5, and 10 cm). These studies revealed that the IJs could find their host at the soil surface as well as down to 10 cm. It indicated that *S. thermophilum* adopts an intermediate (cruising and ambushing) foraging strategy and may be an

ideal biological control agent against insect larvae and their pupae located at varying depths in the field (Ganguly and Gavas 2004a).

2.5. FIELD EVALUATION OF ENTOMOPATHOGENIC NEMATODES

Koch and Bathon (1993) conducted field trials to assess the infectivity of *S. feltiae* and *Heterorhabditis* spp. on coleopteran insects, and reported that only species of the families Carabidae, Staphylinidae, Chrysomellidae, Curculionidae, and Elateridae were affected by the nematode application.

Sosamma and Rashmi (2002) carried out survey of entomopathogenic nematodes in kerala and reported that *H. indica* is distributed throughout Kerala. They found *H.indica* and *Steinernema* spp. were pathogenic to *Oryctes rhinoceros, Rhynchophorus ferrugineus* and *Opisina arenosella*, pests of coconut and *Odoiporus longicollis*, the pseudo-stem borer of banana. Sosamma *et al.* (2002) reported natural occurance of entomogenous nematodes from the body of *O. rhinoceros.*

Martinez *et al*. (2008) studied the efficacy of *S. carpocapsae* on flat-headed root borer *Capnodis tenebrionis* (Coleoptera: Buprestidae) in 5 field trials in the province of Valencia, Spain. The application of EPN was performed during spring and summer into the soil around apricot trees at the rate of 1 and 1.5 million infective juveniles per tree through drip irrigation, injection or by a drench. The control of *C. tenebrionis* larvae was observed to be 75 to 90 per cent.

S. carpocapsae and *H. bacteriophora* were shown to reduce sweet potato weevil, *Cylas formicarius* densities up to 83 and 81 per cent on plants treated with the two species, respectively under field conditions (Jansson *et al.,* 1990).

Several species of cutworms, *Agrotis spp., Spodoptera frugiperda, S. exigua* and *S. litorallis* cause serious problems to agricultural, vegetable and forage crops, worldwide. They are highly susceptible to a number of entomopathogenic nematode species and strains (Morris and Converse, 1991).

Control of *Agrotis segetum,* with *S. feltiae* in lettuce was equivalent to endosulfan (Lossbroek and Theunissen, 1985) under field conditions. *A. ipsilon* has been effectively managed with *S. carpocapsae* on golf course greens. Larvae and pupae of armyworms are very susceptible to entomopathogenic nematodes (Kaya and Grieve, 1982), and can be effectively managed by nematodes. Richter and Fuxa (1990) reported 33-43% infection of *S.frugiperda* by *S. carpocapsae* in field corn. They also found that spraying of nematodes onto corn ears caused up to 71 per cent infection of *S. frugiperda* and they concluded that *S. carpocapsae, S. riobrave,* and *H. megidis* have potential for controlling *S. frugiperda.*

The corn rootworms, *Diabrotica spp.,* are important pests of corn. In North America *D. virgifera* and *D. barberi* are the two dominant species that cause significant economic losses to maize. Nematode applications for rootworm suppression were ineffective in the early experiments (Munson and Helms, 1970), but more recently in field studies, *S. carpocapsae* significantly reduced maize root damage (Ellsbury *et al.,* 1996), reduced rootworm larval population (Jackson, 1996), and rootworm adult emergence (Ellsbury *et al.,* 1996). In some cases, nematode performance was equal to, or better than, insecticides (Wright *et al.,* 1993). The limiting factors of efficacy are the need for timing of application to coincide with the phenology of susceptible stages of *Diabrotica spp.* (Jackson and Brooks, 1995) and the adverse effects of desiccation on survival of the nematodes. Nishimatsu and Jackson, 1998 reported that the combined use of insecticides (tefluthrin) with entomopathogenic nematode may offer an integrated approach for rootworm management.

One of the first insects to be controlled commercially using EPNs was the black currant borer moth, the caterpillars of which bore through the stems of black currants often halving the yield and vitamin-C content of the remaining berries (Bedding and Miller, 1981). Over a million cuttings were stacked and sprayed all over with a concentrated suspension of *S. feltiae* infective juveniles and nearly 100 per cent of the larvae had been killed by nematodes. Several plantations that had serious infestations of currant borer moth were treated by spraying the bushes

with a suspension of *S.feltiae* infective juveniles and this reduced borer moth populations by over 70 per cent (Miller and Bedding, 1982).

Gouge *et al.* (1999) conducted experiment by applying 2.5 billion nematodes/ha (*S. riobrave*) against cotton pink boll worm, *Pectinophora gossypiella* and observed that the numbers of cotton bolls infested by this pest during the season were reduced and the cotton yield increased 19 per cent compared with untreated cotton fields.

The black vine weevil, *Otiorrhynchus sulcatus,* is the major pest of the potted plant industry, worth over $10 billion annually worldwide, and in the larval stage is also one of the most susceptible insects to EPNs (Bedding and Miller, 1981). Suspensions of various *Heterorhabditis* species simply applied to the surface of soil within pots usually results in complete control. Control of this pest around the world provides one of the major markets for EPNs.

EPNs can be manipulated to control even insects that are the least susceptible to them like banana weevil. EPNs cannot penetrate the weevils through the long proboscis or through the anus which is closed like a vice and they cannot get into the breathing holes (spiracles) because these are tightly covered with the wing cases. To overcome this, a three step methodology *viz.,* 1) Using a de suckering tool, cut out a cone of banana tissue from the residual corm. 2) To place in the resulting hole, poly acrylamide gel crystals together with EPNs and 1 per cent mineral oil. 3) To replace the cone of banana tissue. The poly acrylamide gel absorbs sap from the hole which would have stopped the EPNs working and provides a large surface area from which the EPNs can contact the weevils. Replacing the cone encourages attracted weevils to remain. The one per cent oil smears at the edge of the wing covers reducing air intake and the weevil has to lift its wing covers to let air in whereupon EPNs are easily able to enter the now exposed spiracles (Treverrow and Bedding, 1993).

Hussaini *et al.* (2002) studied the indigenous isolates of Steinernema and *H. indica* on brinjal shoot and fruit borer, *Leucinodes orbonalis* and reported that, isolates, PDBC EN 3.1 of *S. bicornutum*, PDBC EN 13.3 of *H. indica* and PDBC EN 6.11 of *S. carpocapsae* recorded maximum mortality of *L. orbonalis* at 50 IJs/larvae in 48 to 72 h of exposure. Preliminary field trial with the isolates, PDBC EN 6.11 of *S. carpocapsae* and PDBC EN 6.71 of *H. indica* on brinjal indicated that higher the concentration of infective juveniles per dose, higher the reduction in borer holes on brinjal fruits and the results were comparable with sprays of neem seed kernel extract. Between the two species evaluated, isolate PDBC EN 6.11 of *S. carpocapse* was more effective in reducing the fruit damage in terms of number of fruits with borer holes and increase in yields.

Umamaheswari *et al.* (2006) tested biocontrol efficacy of EPN on *Spodoptera litura* in blackgram and reported that *Heterorhabditis indica* and *Steinernema glaseri* were found effective at all the doses tested *viz*., 1.25 x 10^9, 2.5 x 10^9 and 5 x 10^9 IJs/ha. Insect mortality increased with increasing dosage levels and exposure time. Maximum mortality was observed with *H. indica* at 5 x 10^9 IJs/ha after 72 h of treatment under glasshouse (75.6%) and microplot (50.6%) conditions. The pod damage was also found minimum with *H. indica* at 5 x 10^9 IJs /ha both under glasshouse (27%) and microplot (34.33%) conditions. *H. indica* was found more effective than *S. glaseri* in the management of *S. litura.*

Bullock and Miller (1994) tested field efficacy of *S. carpocapsae* against *Pachnaeus litus* and *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in citrus orchard. Water suspensions of *S. carpocapsae* (5×10^6) were applied to soil at the base of citrus trees. Weevil reductions during a post-treatment period of 5 to 7 months ranged from 59-83 per cent for *P. litus* and *D. abbreviatus*, respectively.

Abbas *et al*. (2001) evaluated *S. abbasi* and *H. indica* against red palm weevil, *Rhynchophorus ferrugineus*, in United Arab Emirates. Both nematode species were proved pathogenic to larval and adult stages of this insect. At concentrations of 100, 200 and 400 IJs/larva, the rate of mortality caused by *S.*

abbasi were 75, 95 and 100 per cent, respectively, in 3rd instar and 55, 90 and 100 per cent in 5th instar. *H. indica* caused 40, 70 and 100 per cent mortality in 3rd instar and 30, 80 and 100 per cent in 5th instar.

Hussaini *et al.* (2005) evaluated efficacy of four entomopathogenic nematodes (*Heterorhabditis indica*, *H. bacteriophora*, *Steinernema carpocapsae* and *S. abbasi*) against white grubs, *Holotrichia longipennis* (Coleoptera: Scarabaeidae) on turf grass in Srinagar. Nematodes were applied at 5×10^9 IJs/ha and observations on grub mortality were taken at 5-day intervals up to 20 days. Among the isolates tested, *S. carpocapsae* and *S. abbasi* caused 30 to 40 per cent mortality, whereas *H. indica* and *H. bacteriophora* caused 20 to 25 per cent mortality at 10 days after nematode application. White grub population was reduced by 55.7 per cent with *S. carpocapsae*, 53.1 per cent with *S. abbasi*, 42.3 per cent with *Heterorhabditis indica* and 39.6 per cent with *Heterorhabditis bacteriophora*. *Steinernema* spp. reduced grub population more effectively than *Heterorhabditis* spp.

Richard and Scot (1994) applied *H. bacteriophora* through two different methods i.e., aqueous suspension (4.9 billion IJs/ ha) and infected Galleria cadaver (83,700 per ha) against *C. formicarius* in sweet potato field of Tropical Research and Education center, Homestead. The data showed that aqueous suspensions of nematodes were as effective as applications of *G. mellonella* cadavers infected with *H. bacteriophora* for controlling damage by sweet potato weevil to storage roots. Also, they found that persistence of nematodes was similar in both the methods of application.

Abdel Razek and Salama (2013) conducted study on susceptibility of red palm weevil (RPW) to *Photorhabdus luminescens* (bacterial symbiont isolated from *Heterorhabditis bacteriophora* strain HP88) and *Bacillus thuringiensis* spp. *tenebrionis* (*Btt*) strain NB-176 in Egypt and proved that cumulative effect of both bacterial insecticides is highly efficient in controlling red palm weevil.

Skrzecz *et al.* (2012) conducted a study on response of pine weevil to the different species of EPN in Poland. The treatments consists of spraying soil around *Pinus sylvestris* stumps with an aqueous suspension of *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*, *H. downesi*, *H. megidis* at a dose of 3.5 million IJs/1000 ml water per stump. The results of the study proved that all tested nematodes showed ability to parasitize *H. abietis* larvae overwintering in *P. silvestris* stumps. The highest extensivity of parasitizm was observed in *H. abietis* larvae parasitized by *S. carpocapsae* and lowest in *H. megidis*.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation entitled "Entomopathogenic nematodes for the management of cashew stem and root borer, *Plocaederus ferrugineus* L. (Coleoptera: Cerambycidae)" was undertaken in the Department of Agricultural Entomology, College of Horticulture (COH), Kerala Agricultural University (KAU), Thrissur during 2013-14. The field experiment was carried out in the Cashew Research Station, Madakkathara. The materials used and the methodologies adopted are elucidated in this chapter.

3.1. MASS MULTIPLICATION OF WAX MOTH LARVAE IN THE LABORATORY

The EPN spp. were multiplied on greater wax moth (*Galleria mellonella* L.) larvae (Plate 1). The wax moth larvae in turn were mass multiplied on the synthetic diet as described by Singh (1994). The composition of artificial diet is given below

Corn flour - 400 g

Wheat bran - 200 g

Wheat flour - 200 g

Milk powder - 200 g

Yeast - 100 g

Honey - 350 ml

Glycerol - 350 ml

The above different components were mixed in prescribed proportion and used for the rearing of wax moth larvae.

3.2. ENTOMOPATHOGENIC NEMATODE SPECIES

Five species of EPN *viz., Heterorhabditis indica* Poinar, *H. bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser) Wouts, *S. abbasi* Elaward and *S. bicornutum* Tallosi, were used in the study. EPNs were collected from CPCRI, Kayamkulam and BRS, Kannara. These were cultured on the larvae of greater wax moth (*Galleria mellonella* L.) in the lab by following the method as described by Dutky *et al.* (1964). About 10- 15 final instar larvae of greater wax moth larvae were washed in 0.01 per cent formalin and placed in a sterilized standard Petri dish (100 × 15 mm) containing two moist filter papers. The infective juveniles of EPNs were released into this Petri dish at the rate of 50 IJs/ larvae (Plate 2a). After two days dead cadavers (Plate 2b) were washed in water and transferred to a White's trap.

Inverted watch glass lined with Whatman No. 1 filter paper were placed in the small Petri dish. Then the dead cadavers were arranged on it radially. After arranging the cadavers, the small Petri dish was kept in a large Petri dish (150 × 25 mm) which contained sterile water. The filter paper was kept wet by adding 5-10 ml of sterile water around dead larvae daily (Plate 3a to 3g).

The IJs found emerging from cadaver of wax moth larvae migrated into the water were collected and stored in tissue culture flasks at 15ºC in a BOD incubator. The EPN cultures were renewed every month to obtain fresh infective juveniles and used for the various experiments.

3.3. COLLECTION AND MAINTENANCE OF CASHEW STEM AND ROOT BORER GRUBS

3.3.1. Collection of grubs

Cashew stem and root borer (CSRB) grubs were collected from infested cashew trees of Cashew Research Station, Kerala Agricultural University, Madakkathara. Infested cashew trees were identified by observing the external

Plate 1. Mass multiplication of wax moth larvae on artificial diet

Plate 2a. Wax moth larvae inoculated with EPN

Plate 2b. EPN infected wax moth cadaver

Plate 3a. Harvesting of EPN using White's trap method

Plate 3b. Mass multiplication of *S.carpocapsae* on wax moth larvae

Plate 3c. Mass multiplication of *S. abbasi* on wax moth larvae

Plate 3d. Mass multiplication of *S. bicornutum* on wax moth larvae

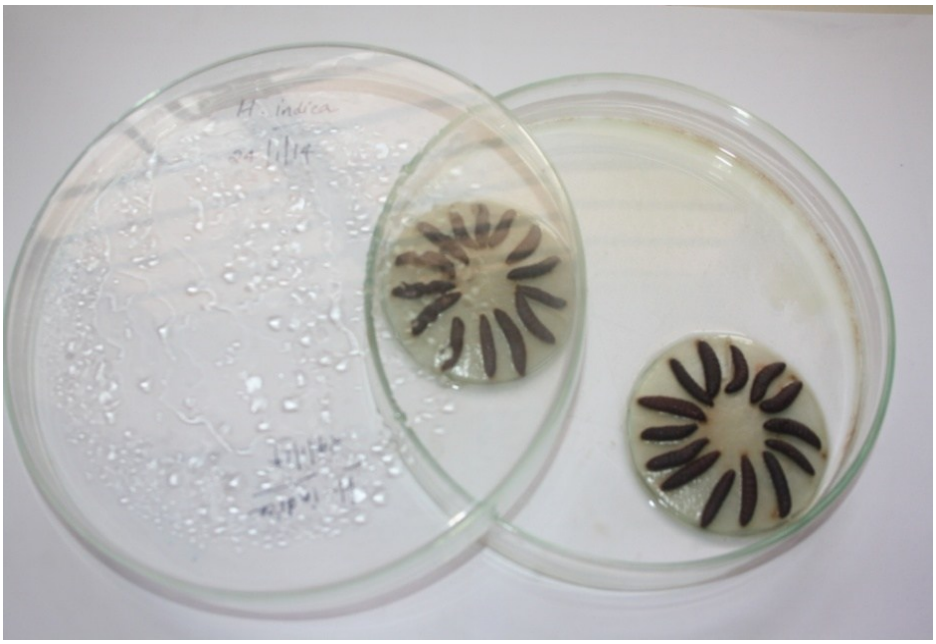

Plate 3e. Mass multiplication of *H. indica* on wax moth larvae

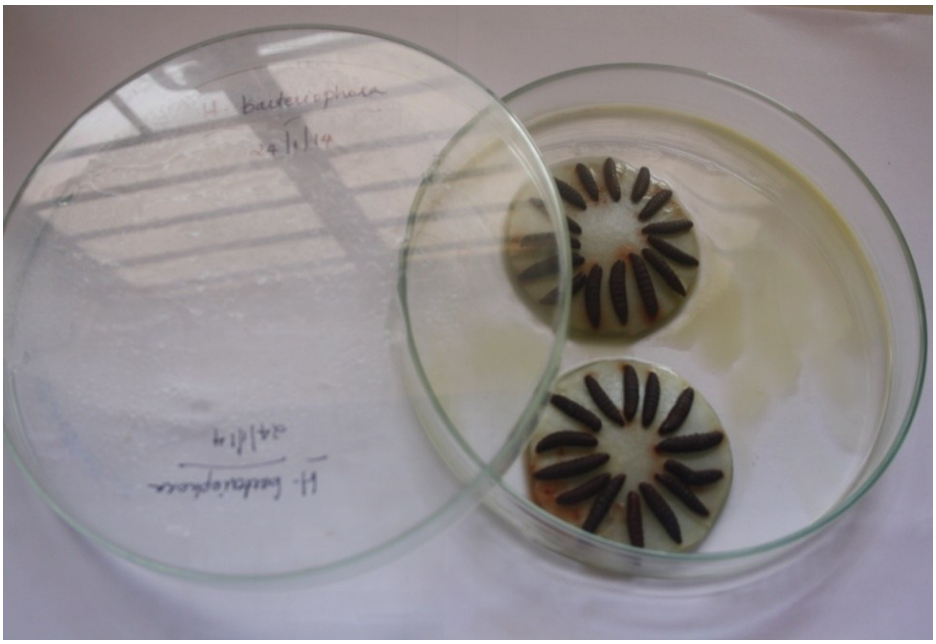

Plate 3f. Mass multiplication of *H. bacteriophora* on wax moth larvae

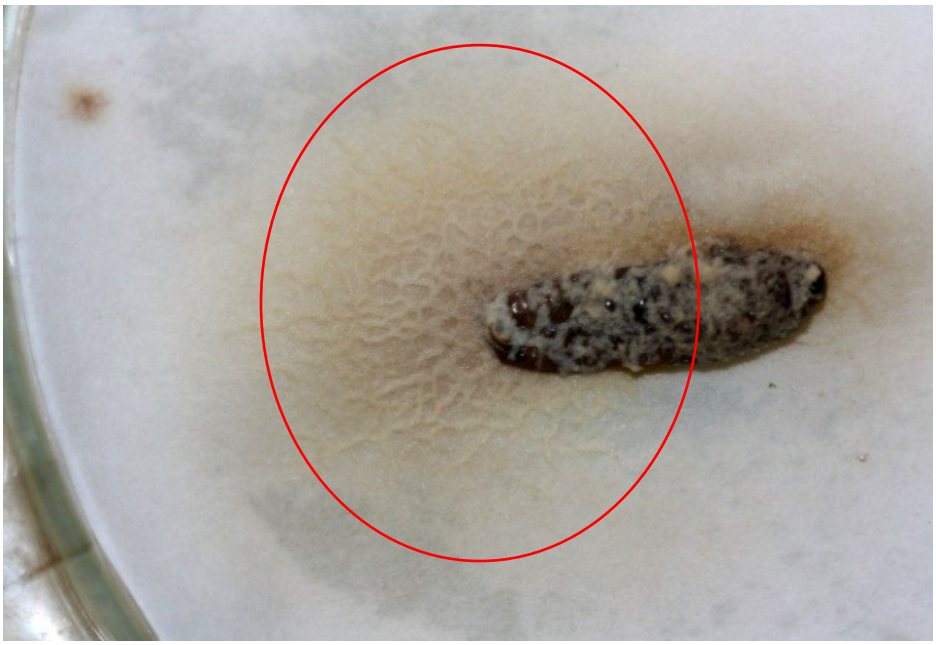

Plate 3g. Emergence of EPN from *Galleria mellonella* larvae

symptoms and the grubs were extracted from the infested portion of the trees by checking along the larval tunnels (Plate 4a to 4d).

3.3.2. Assessing the age of *P. ferrugineus* grubs

The age of the grub was determined by measuring the width of prothoracic shield (PTS) using vernier caliper (Plate 5). Prothoracic shield width of different instars are given below.

Instar level	**Prothoracic shield width(cm)**
1st instar	0.31-0.5
2nd instar	0.51-0.7
3rd instar	0.71-0.9
4th instar	0.91-1.1
5th instar	1.2-1.4

(Raviprasad and Shivarama, 2010)

3.3.3. Maintenance of grubs in the laboratory

Grubs of CSRB extracted from the field were brought to the lab and were maintained in individual plastic jars containing cashew bark pieces as food (Plate 6). The culture thus maintained served as a steady supply of grubs required for conducting the experiment.

3.4. PREPARATION OF NEMATODE SUSPENSION

Nematode suspension with different concentrations 100, 500 and 1000 IJs per ml of sterile water were prepared from EPN suspensions collected from nematode extracting dishes.

Plate 4a. Collection of CSRB grubs

Plate 4b . Yellowing of cashew tree due to CSRB attack

Plate 4c. Symptoms of CSRB infestation

Plate 4d. Tunnel formed by CSRB grub

Plate 5. Measurement of grubs by using vernier calliper

Plate 6. Maintenance of CSRB grubs in bottles

3.5. EVALUATION OF POTENT EPN SPECIES AGAINST *P. ferrugineus*

Three species of Steinernematids *viz*., *Steinernema carpocapsae*, *S. abbasi* and *S. bicornutum* and two species of Heterorhabditids *viz*., *Heterorhabditis indica* and *H. bacteriophora* each at three dosages of 100, 500 and 1000 IJs/ ml were evaluated for their potency against CSRB in the lab. The lab experiment was conducted in CRD with 16 treatments including an untreated control. The treatment details are given below.

T_1 = *Steinernema carpocapsae* at 100 IJs/ml

T_2 = *Steinernema carpocapsae* at 500 IJs/ml

T_3 = *Steinernema carpocapsae* at 1000 IJs/ml

T_4 = *Heterorhabditis indica* at 100 IJs/ml

T_5 = *Heterorhabditis indica* at 500 IJs/ml

T_6 = *Heterorhabditis indica* at 1000 IJs/ml

T_7 = *Steinernema abbasi* at 100 IJs/ml

T_8 = *Steinernema abbasi* at 500 IJs/ml

T_9 = *Steinernema abbasi* at 1000 IJs/ml

T_{10} = *Steinernema bicornutum* at 100 IJs/ml

T_{11} = *Steinernema bicornutum* at 500 IJs/ml

T_{12} = *Steinernema bicornutum* at 1000 IJs/ml

T_{13} = *Heterorhabditis bacteriophora* at 100 IJs/ml

T_{14} = *Heterorhabditis bacteriophora* at 500 IJs/ml

T_{15} = *Heterorhabditis bacteriophora* at 1000 IJs/ml

T_{16} = Control

Each treatment was replicated ten times.

3.5.1. Inoculation of grubs

Third instar CSRB grubs of uniform size and weight were exposed to each treatment by using the standard procedure developed by Koppenhofer and Kaya in 1998 (Plate 7). The mortality of grubs was observed upto 15 days, after inoculating it with different EPN species. The dead grubs were kept for nematode extraction as discussed to confirm pathogenecity (Plate 8).

3.6. FORAGING BEHAVIOUR OF *S. carpocapsae* AND *H. bacteriophora*

The optimum depth upto which the entomopathogenic nematode can access the cashew stem and root borer grubs was determined using the protocols developed by Koppenhofer and Kaya (1998). Vertical plastic columns of 5.5 cm diameter and 30 cm length were used for the experiment. Individual plastic column was covered at both ends with 60×50 mm Petri dishes after filling it with sterilised frass material/sawdust with optimum moisture level. Individual grub of cashew stem and root borer was kept in a wire mesh cage of 20 mesh size to prevent its escape from the experimental area. The grubs were kept at four different depths of 0 cm (surface), 5 cm, 10 cm, and 20 cm in the plastic column. IJs were released at the rate of 1000 IJs/ ml of water at the top of the plastic columns (Plate 9). There were 5 replications for each depth.

The columns were then incubated at 30±2ºC for 120 h and then dismantled to retrieve the infected CSRB grub. The infected grubs were dissected and digested in pepsin enzyme solution to determine the number of IJs established in the haemocoel (Mauleon *et al.,* 1993).

Plate 7a. Inoculation of CSRB grub with EPN

Plate 7b. EPN infected CSRB grub

Plate 8. White's trap method for EPN harvesting from CSRB

Plate 9. Foraging study by using vertical plastic column

The experiment was conducted in CRD with eight treatments as given below

T_1 = *S.carpocapsae* at 0 cm

T_2 = *S.carpocapsae* at 5 cm

T_3 = *S.carpocapsae* at 10 cm

T_4 = *S.carpocapsae* at 20 cm

T_5 = *H.bacteripohora* at 0 cm

T_6 = *H.bacteripohora* at 5 cm

T_7 = *H.bacteripohora* at 10 cm

T_8 = *H.bacteripohora* at 20 cm

3.7. FIELD EVALUATION OF EFFECTIVE EPN SPECIES AGAINST *Plocaederus ferrugineus*

Based on the results of laboratory experiment, *H. bacteriophora* was selected for evaluating its field efficacy against *P. ferrugineus.* Before the application of treatment, the infested trees were selected based on the external symptoms of grub infestation. Application of EPN was done by three different methods *viz*., drenching the soil with EPN suspension (Plate 10 a), placing EPN impregnated sponges in bore holes (Plate 10b) and placing EPN infected wax moth cadavers in the bore holes (Plate 10c).

Plate 10a. Drenching with EPN suspension

Plate 10b. Sponge application in grub infested holes

Plate 10 c. Cadaver application in grub infested holes

Plate 10 d. chlorpyriphos 0.2% application

The experiment was laid out in RBD with five treatments including an untreated control. There were four replications for each treatment.

T_1 = Drenching with *H. bacteriophora* suspension, 500 ml suspension in 5 lit of water (1000 IJs/ ml of water)

T_2 = Placing *H. bacteriophora* impregnated sponges in grub infested holes,10 ml of EPN suspension per each sponge(1000 IJs/ ml)

T_3 = Placing *H.bacteriophora* infested wax moth cadaver in grub infested hole, 3 holes/ tree (10,000-15,000 IJs/ cadaver)

T_4 = Chlorpyriphos 20 EC @ 0.2% swabbing and drenching

T_5 = Absolute control

Drenching of the soil with EPN suspension was done at the rate of 500ml EPN suspension in 5 lit of water/tree (1000IJs/ml of water).One cubic inch dimension sponge was used to apply EPN. Three infested holes on cashew tree were selected and placed one EPN impregnated sponge in each hole. Each sponge contained 10ml of EPN suspension (1000 IJs/ml). EPN infested cadaver of *Galleria mellonella* was placed in grub infested holes. Three infested holes were selected and one infested cadaver was placed in each hole. Each cadaver contained approximately 10,000 to 15,000 EPNs. In treatment four, infested trees were treated with chlorpyriphos 20 EC @ 0.2 per cent as per the KAU POP, 2011 (Plate 10d).

The EPN efficacy was assessed by observing the number of live grubs of *P. ferrugineus* inside the bore holes at one month after treatment application.

Results

4. RESULTS

The results of the study "Entomopathogenic nematodes for the management of cashew stem and root borer, *Plocaederus ferrugineus* L. (Coleoptera: Cerambycidae)" are presented here.

4.1. ASSESSMENT OF THE EFFECTIVENESS OF ENTOMOPATHOGENIC NEMATODES AND DOSE STANDARDIZATION OF EPN INOCULUM.

Effectiveness of the five entomopathogenic nematodes *viz., Heterorhabditis indica* Poinar, *H. bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser) Wouts*, S. abbasi* Elaward and *S. bicornutum* Tallosi against cashew stem and root borer was evaluated by exposing the third instar grubs to different inoculum levels of the nematodes. The results are presented in Table 1.

All the nematodes except *S. abbasi* and *S. bicornutum* recorded hundred per cent mortality at all the three doses evaluated. *S. abbasi* and *S. bicornutum* recorded hundred per cent mortality at both 1000 IJs/ ml as well as 500 IJs/ ml. However, 100 IJs/ ml, recorded lesser mortality of 90 and 30 per cent respectively.

Significant variation was observed among the different nematode species in terms of number of days taken for causing 100 per cent mortality. The mean number of days for 100 per cent mortality varied from 1.5 to 13.4 for different species. *Steinernema carpocapsae* at 1000 IJs/ ml required shorter period of 1.5 days for causing 100 per cent mortality and was significantly superior to all other treatments. *H. bacteriophora* at 1000 IJs/ ml, *S. carpocapsae* at 500 IJs/ ml, *H. indica* at 1000 IJs/ ml and *S. abbasi* at 1000 IJs/ ml required 2.6, 3.2, 3.3 and 3.9 days for acheiving100 per cent mortality and were on par.

Steinernema carpocapsae at 100 IJs/ ml, *H. indica at* 500 IJs/ ml, *S. abbasi* at 500 IJs/ ml and *H. bacteriophora* at 100 IJs/ ml caused 100 per cent mortality of CSRB grubs in 8.1, 8.4, 8.6 and 8.8 days respectively and were on par.

Table 1. Effect of EPN spp. on cashew stem and root borer grubs

Treatment	EPN species	Dose (IJs/ml)	Per cent mortality	Mean days taken for mortality
T_1	*Steinernema carpocapsae*	100	100	8.1 (2.9245)^{e}
T_2	*Steinernema carpocapsae*	500	100	3.2 (1.9131)^{bc}
T_3	*Steinernema carpocapsae*	1000	100	1.5 (1.3963)^{a}
T_4	*Heterorhabditis indica*	100	100	12.9 (3.6588)^{g}
T_5	*Heterorhabditis indica*	500	100	8.4 (2.9784)^{e}
T_6	*Heterorhabditis indica*	1000	100	3.3 (1.9249)^{bc}
T_7	*Steinernema abbasi*	100	90	13.4 (3.7262)^{gh}
T_8	*Steinernema abbasi*	500	100	8.6 (3.0083)^{e}
T_9	*Steinernema abbasi*	1000	100	3.9 (2.0910)^{c}
T_{10}	*Steinernema bicornutum*	100	30	14.4(3.8566)^{h}
T_{11}	*Steinernema bicornutum*	500	100	13.4(3.7261)^{gh}
T_{12}	*Steinernema bicornutum*	1000	100	11.2 (3.4168)^{f}
T_{13}	*Heterorhabditis bacteriophora*	100	100	8.8 (3.0480)^{e}
T_{14}	*Heterorhabditis bacteriophora*	500	100	4.8 (2.2904)^{d}
T_{15}	*Heterorhabditis bacteriophora*	1000	100	2.6 (1.7471)^{b}
T_{16}	Control	-	20	14.8 (3.9112)^{h}

Figures in parentheses are square root transformed values

In columns, means superscripted by a common letter are not significantly different by DMRT (P= 0.05)

par. *S. bicornutum* at 1000 IJs/ ml required 11.2 days for cent per cent mortality and was significantly different from all the treatments.

H. indica at 100 IJs/ ml required 12.9 days to bring complete mortality. Both *S. abbasi* at 100 IJs/ ml and *S. bicornutum* at 500 IJs/ ml took 13.4 days and were at par with each other. S. *bicornutum* at 100 IJs/ ml recorded the highest value of 14.4 days for 30 per cent mortality and was on par with untreated control (14.8 days).

Variation, based on dose of inoculums, was observed within the same species for numbers of days for 100 per cent mortality. All the nematodes required significantly lesser number of days at the highest dose of 1000 IJs/ ml as against the lower dose of 100 IJs/ ml evaluated. For instance, 100 per cent mortality was observed in 3.3 days when CSRB grubs were exposed to *H. indica* at a concentration of 1000 IJs/ml. However, the duration for 100 per cent mortality increased to 8.4 and 12.9 days at inoculum levels of 500 and 100 IJs/ ml.

Steinernema carpocapsae required 1.5, 3.2 and 8.1 mean days for 100 per cent mortality at 1000, 500 and 100 IJs/ ml respectively. While *H. bacteriophora* took 2.6, 4.8 and 8.8 days for causing absolute mortality of CSRB grubs at the inoculum levels of 1000, 500 and 100 IJs/ ml.

The number of days for causing100 per cent mortality increased from 3.9 to 8.6 days when the concentration of IJs decreased from 1000 to 100 IJs/ml through 500 IJs/ ml in case of *S. abbasi*. However *S. bicornutum* induced only 30 per cent mortality at the highest dose of 1000 IJs/ ml even after 15 days of treatment.

Attempts at establishing the pathogenecity of the nematodes by extraction from cadaver of treated grubs met with varied results (Table 2). Nematodes could be extracted from the grubs only in the treatments involving *S. carpocapsae* and *H. bacteriophora*, both at the highest dose of 1000 IJs/ ml (Plate 11). Again, only four grubs each out of 10 grubs inoculated with respective nematodes in each treatment yielded infective juveniles.

Table 2. Pathogenecity of EPN spp. on dead cashew stem and root borer

EPN species	Dose (IJs/ ml)	Dead grubs 15 DAI (No.)	EPN emerged grubs 15 DAI (No.)	Mean no. of EPN emerged 15 DAI
S. carpocapsae	100	10	0	0
S. carpocapsae	500	10	0	0
S. carpocapsae	1000	10	4	47,500
H. indica	100	10	0	0
H. indica	500	10	0	0
H. indica	1000	10	0	0
S. abbasi	100	09	0	0
S. abbasi	500	10	0	0
S. abbasi	1000	10	0	0
S. bicornutum	100	02	0	0
S. bicornutum	500	10	0	0
S. bicornutum	1000	10	0	0
H. bacteriophora	100	10	0	0
H. bacteriophora	500	10	0	0
H. bacteriophora	1000	10	4	55,000

DAI – Days after inoculation

Plate 11. Emergence of EPN from the body of CSRB grub

The population of IJs obtained during extraction also showed variation among the two species (Table 2). While *H. bacteriophora* yielded an average of 55,000 IJs/ grub, *S. carpocapsae* yielded only 47,500 IJs/ grub.

4.2. STUDIES ON THE FORAGING BEHAVIOUR OF *H. bacteriophora* and *S. carpocapsae*

The most effective EPNs viz., *S. carpocapsae* and *H. bacteriophora* both at 1000 IJs/ ml, based on their effectiveness were selected for studying their foraging behaviour.

Cashew stem and root borer grubs placed at the surface of the plastic colums recorded infection in case of both *S.carpocapsae* and *H.bacteriophora* as confirmed by foraging test (Table 3). However, grubs placed at 5, 10 and 20 cm depths were not infected in case of *S. carpocapsae*. *H. bacteriophora* caused 100 per cent infection of grubs placed at 0 and 5 cm depth. The grubs placed at 10 cm depth recorded 60 per cent infection, but there was no infection of grubs placed at 20 cm depth.

Heterorhabditis bacteriophora proved to be superior to *S. carpocapsae* in the ability of dauer larvae to establish in the CSRB grubs. A higher number of 20.26 per cent of *H.bacteriophora* juveniles successfully invaded the CSRB grubs placed at the surface level, as against 10.6 per cent recorded in case of *S. carpocapsae.* At 5 and 10 cm depth, 13.36 and 3.36 per cent of infective juveniles of *H.bacteriophora* invaded the CSRB grubs offered respectively (Plate 12).

Table 3. Per cent EPN penetrated the grubs of CSRB

Treatment	EPN species	Depth of column (cm)	Per cent infection	Percentage of EPN penetrated
T_1	*S.carpocapsae*	0	100	10.60
T_2	*S.carpocapsae*	5	-	-
T_3	*S.carpocapsae*	10	-	-
T_4	*S.carpocapsae*	20	-	-
T_5	*H.bacteriophora*	0	100	20.26
T_6	*H.bacteriophora*	5	100	13.36
T_7	*H.bacteriophora*	10	60	03.36
T_8	*H.bacteriophora*	20	-	-

Plate 12. Emergence of EPN from the dissected grubs of CSRB

4.3. FIELD EVALUATION OF EPN SPECIES AGAINST CSRB

Heterorhabditis bacteriophora, which proved to be the most efficient nematode in preceding studies, was selected for the field evaluation.

Infested trees with atleast one live grub (as judged by fresh frass) were selected for imposing the treatments. The results are presented in Table 4. Among the different treatments, application of chlorpyriphos 20 EC (0.2 per cent at 10 ml/l) noticed to be the most effective, recording mean number of 0.25 live grubs per tree one month after the treatment. This was followed by cadaver application, which recorded 0.5 live grubs per tree. Both drenching and sponge application recorded similar values at 1 grub per tree. Control recorded the highest number of 1.5 grub/ tree.

Table 4. Field efficacy of *H. bacteriophora* on cashew stem and root borer

Treatment	Live grubs per tree (Mean number)
T_1- Drenching with *H. bacteriophora*	1.00
T_2- Sponge application with *H. bacteriophora*	1.00
T_3- Cadaver application with *H. bacteriophora*	0.50
T_4- Chlorpyriphos 20 EC (0.2 per cent at 10 ml/l) application	0.25
T_5- Control	1.50

Discussion

5. DISCUSSION

A study was undertaken at College of Horticulture, Vellanikkara, Kerala Agricultural University to determine the efficacy of five different species of entomopathogenic nematodes (EPN) for the management of cashew stem and root borer (CSRB). The foraging behaviour and field efficacy of EPNs were also investigated during the present study. The detailed discussion of the results obtained in the study is presented hereunder.

5.1. ASSESSMENT OF THE EFFECTIVENESS OF ENTOMOPATHOGENIC NEMATODES AND DOSE STANDARDIZATION OF EPN INOCULUM.

The experiment on effectiveness of EPN against cashew stem and root borer showed that all the nematodes except *S. abbasi* and *S. bicornutum* recorded 100 per cent mortality at all the three doses evaluated. Both *S. abbasi* and *S. bicornutum* recorded lower mortality of 90 and 30 per cent respectively at the dose of 100 IJs/ ml (Fig. 1).

Pathogenecity of entomopathogenic nematodes against CSRB have been reported by several workers. For instance, Madhu (1989), who studied the efficacy of entomopathogenic nematode DD-136 (*Neoplectana carpocapse* Weiser, 1955) on cashew stem and root borer (CSRB) reported that the nematode induced maximum mortality in first and second instar grubs.

Vasanthi and Raviprasad (2012) also reported that three EPN species namely, *H. indica, S. bicornutum* and *S. abbasi* could successfully infect cashew stem and root borer grub in the laboratory. Similarly, Gavas (2012) had reported that use of *H. bacteriophora* at 1000 IJs/ ml resulted in 100 per cent mortality of fifth instar CSRB grubs within 48-72 hours. The present study is in conformity with the above reports, underlining the potential of EPN in the management of CSRB.

However, there was variability in pathogenecity of different nematode species against the third instar grubs of CSRB. Several studies have recorded

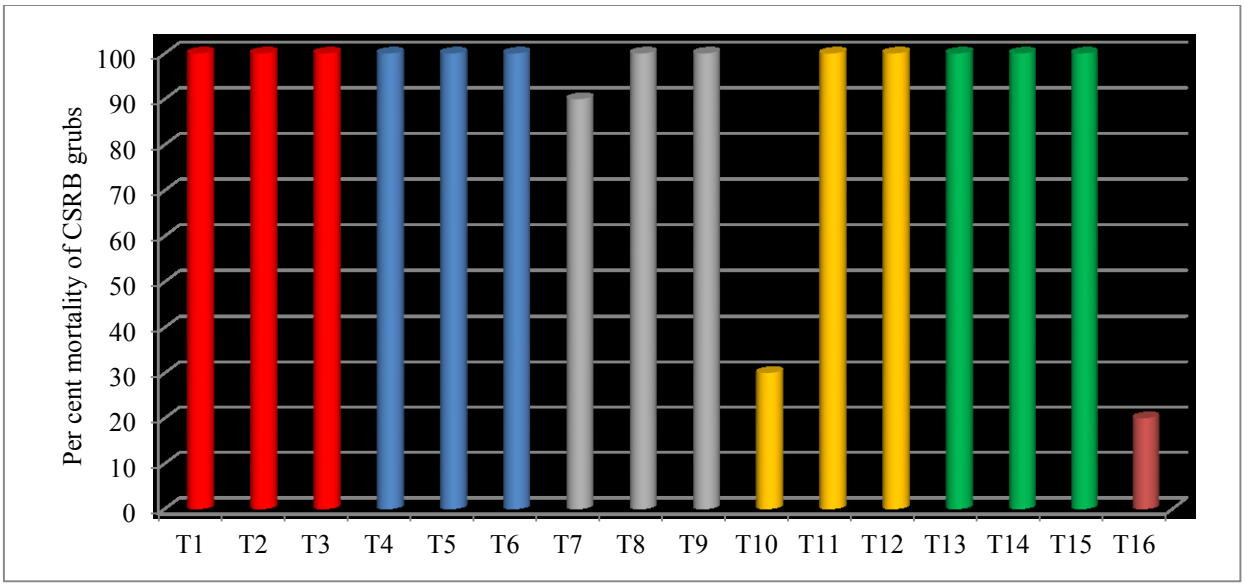

Fig 1. Per cent mortality of cashew stem and root borer grubs

T_1, T_2, T_3 — *S. carpocapsae* @ 100 IJs/ml, 500 IJs/ml, 1000 IJs/ml

T_4, T_5, T_6 — *H. indica* @ 100 IJs/ml, 500 IJs/ml, 1000 IJs/ml

T_7, T_8, T_9 — *S. abbasi* @ 100 IJs/ml, 500 IJs/ml,1000 IJs/ml

T_{10}, T_{11}, T_{12} — *S. bicornutum* @100 IJs/ml 500 IJs/ml, 1000 IJs/ml

T_{13}, T_{14}, T_{15} — *H. bacteriophora* @ 100 IJs/ml, 500 IJs/ml, 1000 IJs/ml

T_{16} = Control

similar variation among EPN to a common host. Stark and Lacey (1999) evaluated the susceptibility of western cherry fruit fly (Diptera;Tephritidae) to different species of entomopathogenic nematodes and reported that *S. carpocapsae* and *H. bacteriophora* were much effective compared to *S. riobrave* and *S. feltiae*. Vasanthi and Raviprasad (2012) observed that Steinernematids recorded higher virulence against CSRB when compared to *H. indica.*

There was significant variation among the different treatments in terms of number of days taken for registering 100 per cent mortality (Fig. 2). The mean number of days taken for causing 100 per cent mortality was varied from 1.5 to 13.4 for different species. *S. carpocapsae* at 1000 IJs/ ml recorded the lesser number of 1.5 days for achieving 100 per cent mortality and was significantly superior over other treatments. *H. bacteriophora* at 1000 IJs/ ml, *S. carpocapsae* at 500 IJs/ ml, *H. indica* at 1000 IJs/ ml and *S. abbasi* at 1000 IJs/ ml required 2.6, 3.2, 3.3 and 3.9 days for 100 per cent mortality and were on par.

Heterorhabditis indica at 100 IJs/ ml required 12.9 days to bring complete mortality. Both *S. abbasi* at 100 IJs/ ml and *S. bicornutum* at 500 IJs/ ml took 13.4 days and they were on par with each other. *S. bicornutum* at 100 IJs/ ml recorded the highest value of 14.4 days for 30 per cent mortality and was on par with untreated control (14.8 days).

The correlation between duration and per cent kill at higher levels of inoculums suggests a dose dependent mortality relationship. Madhu (1989), who evaluated DD-136 nematode at varying dose of 75-200 IJs/ml reported that there was no mortality of third, fourth and fifth instar CSRB grubs, whereas Gavas (2012) reported mortality of fifth instar grubs in 1-2 days at 10,000 IJs/ ml, confirming the dose dependent relationship brought out in this study.

Confirmation of pathogenecity by extraction of nematodes from cadavers of the grubs was partially successful, with 40 per cent of grubs inoculated with *S.*

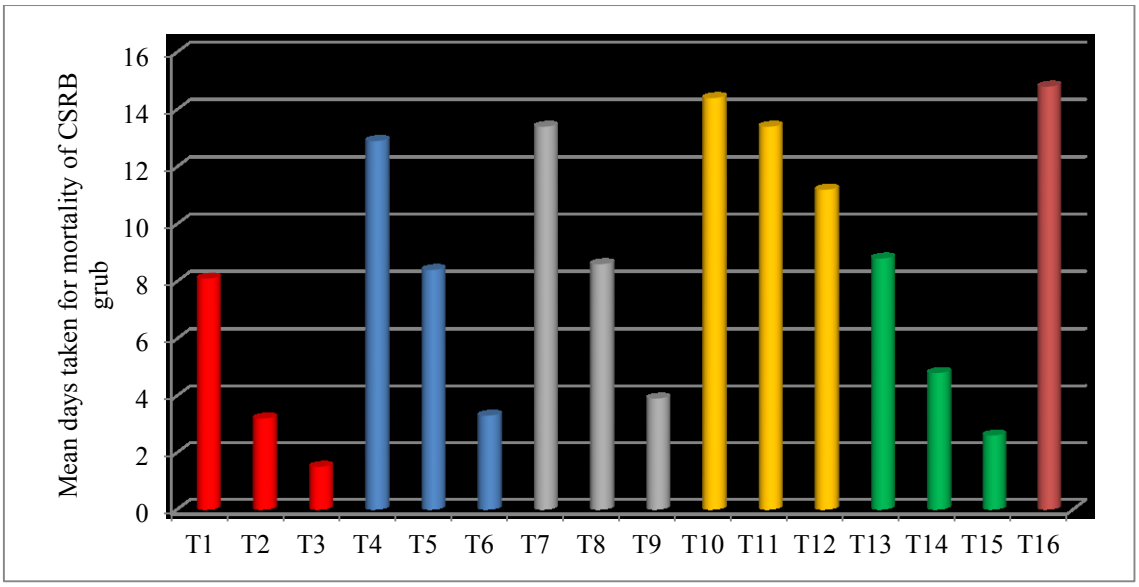

Fig 2. Period taken for causing mortality of cashew stem and root borer grub

T_1, T_2, T_3 — *S. carpocapsae* @ 100 IJs/ml, 500 IJs/ml,1000 IJs/ml

T_4, T_5, T_6 — *H. indica* @ 100 IJs/ml, 500 IJs/ml, 1000 IJs/ml

T_8 — *S. abbasi* @ 100 IJs/ml,

T_9 — 500 IJs/ml,1000 IJs/ml

T_{10}, T_{11}, T_{12} — *S. bicornutum* @100 IJs/ml 500 IJs/ml, 1000 IJs/ml

T_{13}, T_{14}, T_{15} — *H. bacteriophora* @ 100 IJs/ml, 500 IJs/ml, 1000 IJs/ml

T_{16} = Control

carpocapsae and *H.* bacteriophora at 1000 IJs alone yielding IJs during extraction. While the external symptoms like colour of the cadaver and level of secondary decomposition were evident, the non emergence of dauer nematodes from most of the cadavers requires further investigation.

The population of IJs obtained showed variation among the two species (Fig 3). *H. bacteriophora* yielded an average of 55,000 IJs/ grub, whereas *S. carpocapsae* yielded only 47,500 IJs/ grub. These observations were in conformity with the earlier studies on reproductive potential of EPNs by Isik and Nurdan, 2003, who reported that reproductive potential of *Heterorhabditis* spp. was higher than that of *Steinernema* spp. due to its hermaphroditic life cycle.

Boff *et al*., (2000) had reported that as the size of the host increased, the number of IJs obtained per host also increased. A single cadaver of wax moth larva has been known to yield 10,000 – 15,000 infective juveniles. The higher average of 47,500 IJs per CSRB grub in case of *S. carpocapsae* and 55,000 IJs per grub in case of *H. bacteriophora* obtained in the present study is in line with the above findings, considering the much larger size of third instar CSRB grubs as against the wax moth larva. Similar findings have also been reported by Dutky *et al*. (1964); Blinova and Ivanova (1987); Shapiro Di- Ilan and Gaugler (2002).

5.2. STUDIES ON THE FORAGING BEHAVIOUR OF *H. bacteriophora* and *S. carpocapsae*

Foraging parameters such as depth of EPN penetration and the per cent infection of the *S. carpocapsae* and *H. bacteriophora,* which were found to be most effective treatments in the experiment on pathogenecity of EPNs were studied and the results are discussed as follows.

Cashew stem and root borer grubs placed at the surface of the plastic columns recorded infection by both *S.carpocapsae* and *H.bacteriophora.* However, grubs placed at 5, 10 and 20 cm depth were not infected in case of *S. carpocapsae*. Grubs placed at 0 and 5 cm depth recorded 100 per cent infection in

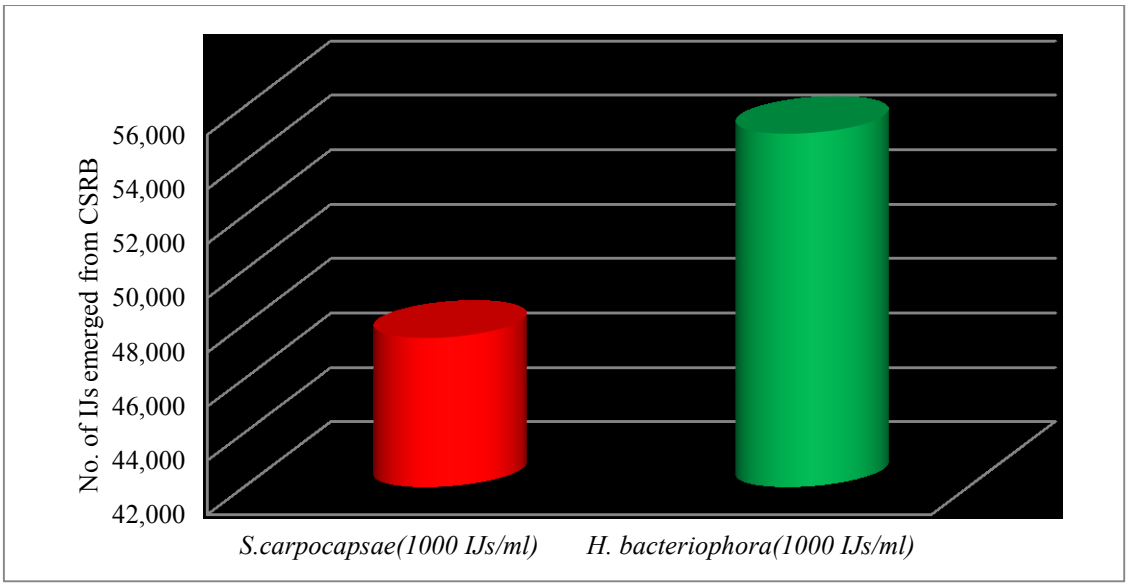

Fig 3. Emergence of EPN from dead grubs of CSRB

case of *H. bacteriophora*. Grubs placed at 10 cm depth recorded 60 per cent infection, while there was no infection of grubs placed at 20 cm depth (Fig. 4).

The above results are in conformity with earlier findings on the foraging nature of two nematode species. Campbell and Gaugler (1993), who evaluated foraging nature of *S. carpocapsae* and *H. bacteriophora* reported that *S. carpocapsae* infected more insects on the surface, while cruising predators like *H. bacteriophora* infected insects that live deep in the soil. Similar results were also reported by Moyle and Kaya (1981), who observed that *S. carpocapsae* act as ambushers which stay near the surface without dispersing far and exhibit nictating behaviour to gain access to hosts that pass by. *H. bacteriophora,* on other hand, was a cruiser, actively searching for its host. The inability of *S. carpocapsae* to penetrate deep into the soil has also been reported by Koppenhoffer and Kaya (1998), who reported that *S. carpocapsae, S. monticolum* and *S. rarum* could not infect the host at 10 cm depth.

The per cent EPN that invaded and established inside CSRB grub also showed difference among the two species (Fig. 5). In case of *S.carpocapsae,* 10.6 per cent infective juveniles could successfully colonize the grubs placed at the surface. The corresponding figure for *H. bacteriophora* was higher at 20.26 per cent. The per cent infective juveniles of *H.bacteriophora* that could invade CSRB grubs at the depths of 5 and 10 cm were 13.36 and 3.36 respectively. But, no IJs of *H.bacteriophora* could be observed from the body of CSRB grubs which were placed at 20 cm depth.

Steinernema carpocapsae was infective only to grubs placed at the surface and the establishment of IJs in the haemocoel of CSRB was also less compared to *H. bacteriophora* which could successfully infect a host located at surface or up to 10 cm depth. This confirmed the cruising foraging behaviour of *H. bacteriophora*. As the depth increased the number of IJs established in the haemocoel of the grubs were observed to be less. These observations were in agreement with the earlier studies on foraging behaviour of *S. glaseri* (Ganguly and Gavas, 2004b)

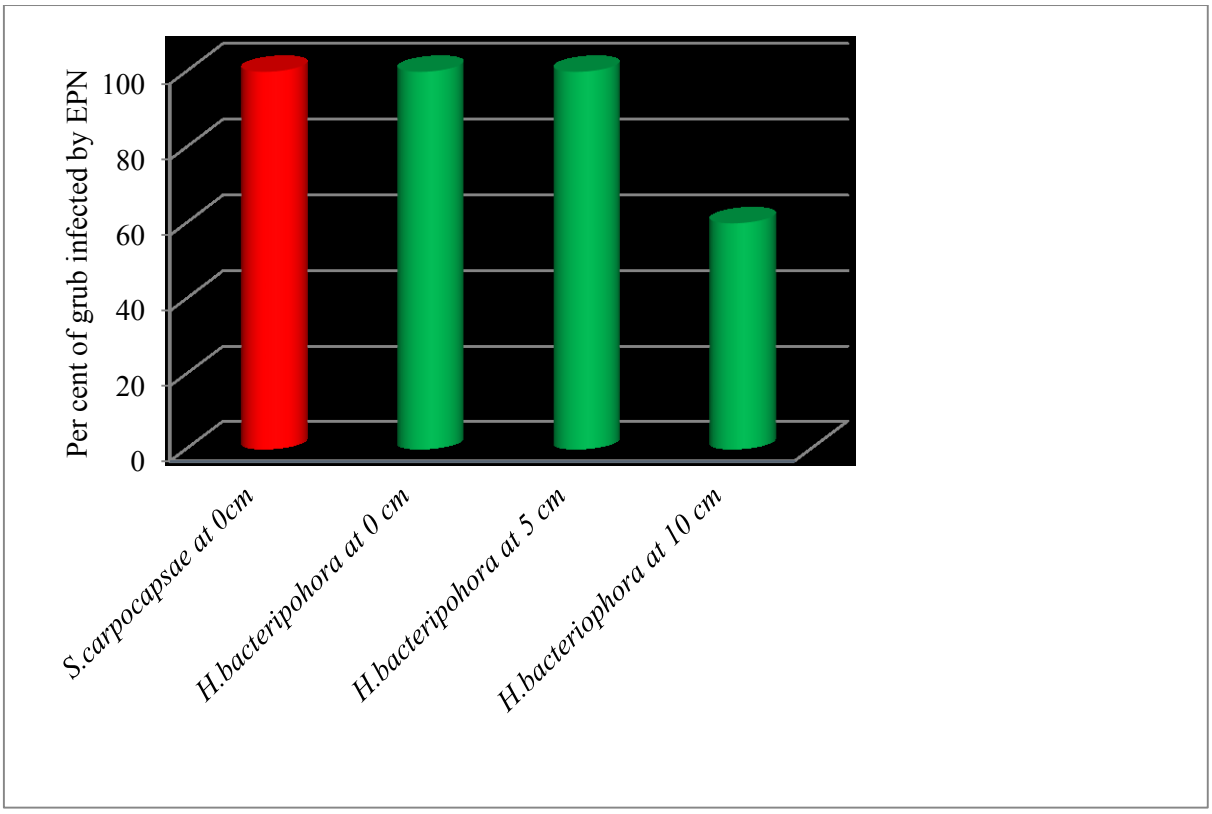

Fig 4. Per cent CSRB grubs infected by EPN

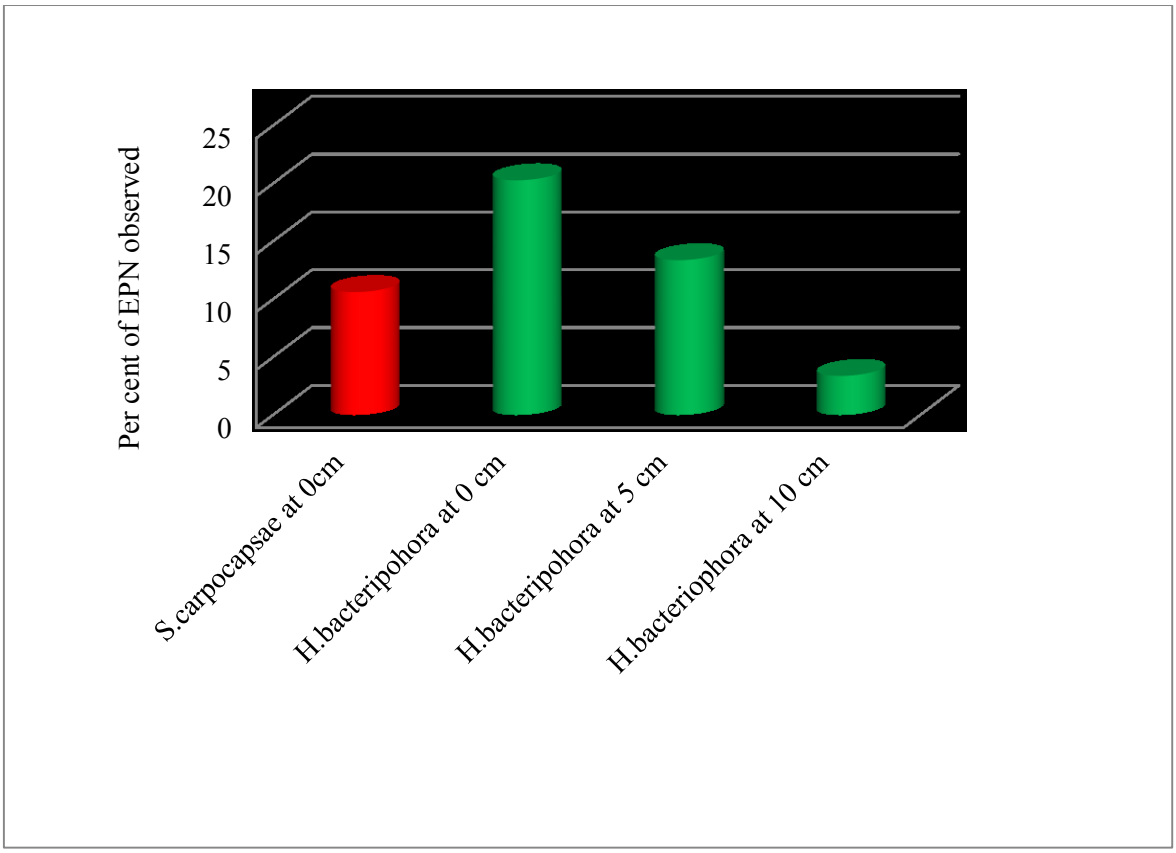

Fig 5. Per cent EPN observed in haemocoel of CSRB

who reported that as the depth increased, the establishment of IJs in the body of the host decreased.

5.3. FIELD EVALUATION

The efficacy of the *H. bacteriophora,* identified as the most effective species based on pathogenecity and foraging studies was evaluated in the field. Application of EPN was done by using three different methods namely drenching the soil with EPN suspension, sponge application and cadaver application.

Chlorpyriphos was observed to be the most effective of all treatments, recording mean number of 0.25 live grubs per tree, followed by cadaver application, which recorded 0.5 live grubs per tree. Both drenching and sponge application recorded similar values of one grub per tree. The highest number of 1.5 grub/ tree was recorded in control.

The results indicated that there is a possibility of cadaver treatment in managing cashew stem borer grubs. Field studies on efficacy of EPN against CSRB have hardly been reported. Hence confirmation of the above results is required through further studies.

EPN have been reported as effective against a number of coleopteran pests such as root grubs in cardamom (AICRPS, 2011), red palm weevil in coconut (Saleh, 2011) and sweet potato weevil (Jansson, 1990). Allard and Moore (1989) as well as Bedding and Miller (1981) proved that *Heterorhabditis spp.* caused mortality of adult and grubs of coffee berry borer, *Hypothenemus hampei* and black vine weevil, *Otiorrhynchus sulcatus* respectively under field conditions.

The present investigations confirmed the potential of entomopathogenic nematodes for the management of cashew stem and root borer. Given the cryptic nature of the pest, *H. bacteriophora,* with the cruising foraging nature might be a better choice as a biocontrol agent against CSRB, as indicated in the foraging

studies. However development and standardization of mechanism to deliver the EPN at a point close to the grubs need to be evolved.

Summary

6. SUMMARY

The present study entitled "Entomopathogenic nematodes for the management of cashew stem and root borer, *Plocaederus ferrugineus* L. (Coleoptera: Cerambycidae)''was undertaken in the Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Thrissur during 2013-14. The field experiment was carried out in the Cashew Research Station, Madakkathara. The experiment was carried out to evaluate the possibility of utilization of five different species of entomopthogenic nematodes *i.e., Heterorhabditis indica* Poinar, *H. bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser) Wouts*, S. abbasi* Elaward and *S. bicornutum* Tallosi, to control the cashew stem and root borer (CSRB) *Plocaederus ferrugineus* L.

The objectives of the study were to identify the potent species of entomopathogenic nematode (EPN) and to determine the optimum inoculum levels needed to induce mortality, analyze the foraging behaviour of EPN species and to test the field efficacy of the selected EPN species against CSRB.

Five species of EPN were obtained from CPCRI, Kayamkulam and BRS, Kannara. These EPNs were multiplied on greater wax moth (*Galleria mellonella*) larvae following the method described by Dutky *et al.* (1964). The wax moth larvae in turn were mass multiplied on the synthetic diet as described by Singh (1994).

Cashew stem and root borer (CSRB) grubs were collected from infested cashew trees at Cashew Research Station, Kerala Agricultural University, Madakkathara. These grubs were brought to the lab and were maintained in individual plastic jars containing cashew bark pieces as food. The culture thus maintained served as a steady supply of grubs required for conducting the experiment. The third instar grubs were selected based on the width of prothoracic shield (PTS) using vernier caliper (Raviprasad and Shivarama, 2010).

All the five EPNs were tested each at three different inoculum levels against the grubs of CSRB in the laboratory. Third instar grubs of CSRB were introduced into Petri dishes lined with Whatman No.1 filter paper and were inoculated with different species of EPN at three different inoculum levels (100, 500 and 1000 IJs/ml distilled water). The mortality of grubs was recorded up to 15 days of treatment. Complete mortality was observed in all the treatments except in case of grubs which were treated with *S. abbasi* and *S. bicornutum* at 100 IJs/ ml. *S. carpocapsae* showed significant difference from other treatments at 1000 IJs/ ml and it was followed by *H. bacteriophora.* Though mortality of grubs was observed in all species of EPN at different inoculum levels, emergence of EPN was observed only from those grubs treated with *H.bacteriophora* and *S. carpocapsae* both at 1000 IJs/ml. In both the species, EPN emerged from four grubs out of 10 grubs. The population of nematodes showed variation among the two species (Table 2). While *H. bacteriophora* yielded an average of 55,000 IJs/ grub, whereas *S. carpocapsae* yielded only 47,500 IJs/ grub.

Based on the results of first experiment, out of the five EPN species, only *S.carpocapsae* and *H.bacteriophora* were selected for further studies on their foraging behaviour. The optimum depth upto which the entomopathogenic nematode can access the cashew stem and root borer grubs was determined by using the protocols developed by Koppenhofer and Kaya (1998). Cashew stem and root borer grubs placed at the surface recorded infection in case of both *S.carpocapsae* and *H.bacteriophora.* However, grubs placed at 5, 10 and 20 cm depths were not infected in case of *S. carpocapsae*. Grubs placed at 0 and 5 cm depths recorded 100 per cent infection in case of *H. bacteriophora*. Grubs placed at 10 cm depth recorded 60 per cent mortality while there was no infection of grubs placed at 20 cm depth.

In case of *S.carpocapsae,* 10.6 per cent infective juveniles could successfully invade the body of CSRB grubs. In case of *H.bacteriophora*, 20.26 per cent infective juveniles could successfully colonize the body of CSRB grubs which were placed on the surface.

The per cent infective juveniles of *H.bacteriophora* could penetrate CSRB grubs at the depth of 5 and 10 cm was 13.36 and 3.36 respectively. However, *H.bacteriophora,* IJs could not be observed from the body of CSRB grubs which were placed at 20 cm depth.

Based on the results of laboratory experiment, *H. bacteriophora* was selected for evaluating its field efficacy against CSRB. The infested trees were selected based on the external symptoms of grub infestation. Application of *H. bacteriophora* was done by using three methods, *viz.,* drenching the soil with EPN suspension, placing EPN impregnated sponges in bore holes and placing EPN infected wax moth cadavers in the bore holes. Chemical treatment was also done by using chlorpyriphos 20 EC at 10 ml/l (KAU POP, 2011).

Chlorpyriphos was observed to be the most effective, recording mean number of 0.25 live grubs per tree, followed by cadaver application, which recorded 0.5 live grubs per tree. Both drenching and sponge application recorded similar values at 1 grub per tree. The highest number of 1.5 grub/ tree were recorded in control.

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ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF CASHEW STEM AND ROOT BORER, *Plocaederus ferrugineus* L. (COLEOPTERA: CERAMBYCIDAE)

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ABSTRACT OF THE THESIS

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ABSTRACT

The study entitled "Entomopathogenic nematodes for the management of cashew stem and root borer, *Plocaederus ferrugineus* L. (Coleoptera: Cerambycidae)''was conducted at the Cashew Research Station, Madakkathara, Kerala Agricultural University during 2013-14 in order to evaluate the possibility of utilization of five different species of entomopthogenic nematodes *i.e., Heterorhabditis indica* Poinar, *H. bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser) Wouts*, S. abbasi* Elaward and *S. bicornutum* Tallosi, to control the cashew stem and root borer (CSRB) *Plocaederus ferrugineus* L.

The objectives of the study were to identify the potent species of entomopathogenic nematode (EPN) and to determine the optimum inoculum levels needed to induce mortality, analyze the foraging behaviour of EPN species and to test the field efficacy of the selected EPN species against CSRB.

The five species of entomopathogenic nematodes were tested each at three different inoculum levels against the grubs of CSRB in the laboratory. Third instar grubs of CSRB were introduced into petridishes lined with Whatman No.1 filter paper and were inoculated with different species of EPN at three different inoculum levels (100, 500 1000 IJs/ml of distilled water). The mortality of grubs was recorded up to 15 days of treatment. Complete mortality was observed in all the treatments except in case of grubs which were treated with *S. abbasi* and *S. bicornutum* at 100 IJs/ ml. *Steinernema carpocapsae* showed significant difference from other treatments at 1000 IJs/ ml and it was followed by *H. bacteriophora.* Though mortality of grubs was observed in all species of EPN at different inoculum levels, emergence of EPN was observed only from those grubs treated with *H.bacteriophora* and *S. carpocapsae* both at 1000 IJs/ml inoculum level.

Based on the results of first experiment, out of the five EPN species, only *S.carpocapsae* and *H.bacteriophora* were selected for further studies on their foraging behaviour. Foraging behavior was tested using vertical plastic columns of 5.5 cm diameter and 30 cm length as per the method developed by Koppenhofer and Kaya (1998). Individual grub of CSRB was placed in a wire mesh cage at 0, 5, 10 and 20 cm depth in the plastic column and both ends were covered with Petridishes (60×15 mm). One ml of EPN suspension containing 1000 IJs was released from

the top of the plastic columns and incubated for 120 hours.

Heterorhabditis bacteriophora could penetrate up to a depth of 10 cm within a time of 120 hours, while *S. carpocapsae* could only infect the grubs which were placed on the surface. This indicates that *H. bacteriophora* has better foraging capacity as compared to *S. carpocapsae*.

Based on the results of laboratory experiment, *H. bacteriophora* was selected for evaluating its field efficacy against CSRB. Application of *H. bacteriophora* was done by using three methods, *viz*., drenching the soil with EPN suspension, placing EPN impregnated sponges in bore holes and placing EPN infected wax moth cadavers in the bore holes. Chemical treatment was also done by using chlorpyriphos 20 EC @ 0.2 per cent (KAU POP, 2011). Live grubs were observed in all treatments after one month of treatment application. Chlorpyriphos has performed better compared to the EPN treatments.