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Chapter 12

Entomopathogenic Nematodes in Pest Management

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1. Introduction

Plantation crops are perennial in nature, cultivated extensively in tropics/subtropics. The important plantation crops in India are coffee, tea, rubber, cashew, cocoa, coconut, arecanut, oil palm and intercrops like black pepper, cardamom, ginger and turmeric, which are not the exception to be damaged by insect pests at all life stages. Insect pests cause damage not only to standing crops but seedlings in nursery and seeds during storage. By virtue of quick knock down effect, chemical insecticides are the preferred options for these insect pests. But increased awareness on the side effects caused by indiscriminate use of chemical pesticides and global concern regarding possible traces of pesticides residues in export plantation commodities had made eco-friendly integrated pest management (IPM) the need of the present era. Among the various components of IPM, the most efficient tool for ecological sustainability is the biological pest suppression. Biological control agents broadly involve parasitoids, predators and entomopathogens like bacteria, fungi and nematodes which cause lethal infections. The nematodes which are not parasitic on plants but infect insects and used as biological control agents in insect pest management are known as entomopathogenic nematodes (EPN). Using EPN as a component of IPM will aid to decline the dependence on insecticides and promotes eco-friendly pest management techniques in plantation crops as they are safe for plants and animals. They recycle and persist in environment and highly host specific. EPN play a vital role in the bio-suppression of various agricultural pests in particularly soil pests (root grubs) of plantation crops have been investigated and effectively utilized in the case of coconut, arecanut, cashew and cardamom pests

(Sosamma 2003; Varadarasan *et al.*, 2006; Vasanthi, 2012). Some of the important plantation crops and their pests are given in Table 12.1.

Table 12.1: Pest Attacking some Important Plantation Crops

Name of the Crop	*Important Pests*
Coconut	Rhinoceros beetle (*Oryctes rhinoceros* L.)
	Red palm weevil (*Rhynchophorus ferrugineus* F.)
	Leaf eating caterpillar (*Opisina arenosella* Walker)
	White grub (*Leucopholis coneophora* Burm.)
Arecanut	White grub (*Leucopholis* sp.)
	Spindle bug (*Carvalhoia arecae* Miller and China)
Oil palm	Rhinoceros beetle (*Oryctes rhinoceros* L.)
	Red palm weevil (*Rhynchophorus ferrugineus* F.)
Cashew	Stem and root borer (*Plocaederus* sp. and *Batocera rufomaculata* De Geer)
	Leaf miner (*Conopomorpha syngramma* M.)
Cardamom	Root grub (*Basilepta fulvicorne* Jacoby)
	Capsule borer (*Conogethes punctiferalis* Guen.)
	Cardamom thrips (*Sciothrips cardamomi* Ramk.)
Tea	Pale mite (*Acaphyllisa parindiae*)
	Scarlet mite (*Brevipalpus australis*)
	Cut worm (*Spodoptera litura*)
Coffee	Coffee berry borer (CBB), *Hypothenemus hampei*

The interest in the use of EPNs as biological control agents for control of insect pests has increased exponentially over the past decades. A decade ago, the idea of using nematodes to control pest populations was vague promise held by handful of researchers working with these obscure insect parasites. Today, they are no longer a laboratory curiosity but have begun to gain acceptance as environmentally benign alternatives to chemical insecticides. However, their potential as IPM component has been realized recently. These nematodes have been widely used to treat insect pest problems in agriculture, horticulture, plantation and forestry crops in India and abroad (Hussaini *et al.*, 2003; Banu and Rajendran 2002; Grewal *et al.*, 2004). The ease of mass production and exemption from registration requirements are the two major reasons for early interest in the commercial developments of EPNs and are now commercially mass-produced in six of the seven continents.

2. Entomopathogenic Nematodes

Entomopathogenic nematodes (Families Heterorhabditidae and Steinernematidae) are soft bodied, non-segmented roundworms similar in morphology to plant parasitic nematodes that are obligate or sometimes facultative parasites causing death to insects known as entomopathogenic nematodes (EPNs). They occur naturally in soil environments and locate their host in response to carbon dioxide, vibration and other chemical cues (Kaya and Gaugler, 1993), have been

described from 23 nematode families (Koppenhofer, 2007). Of all of the nematodes studied for biological control of insects, the steinernematids and heterorhabditids are produced commercially and used as bio-agents, because, they possess many of the attributes of effective biological control agents (Kaya and Gaugler, 1993; Grewal *et al.*, 2005a; Koppenhofer, 2007) and have been utilized as classical, conservational, and augmentative biological control agents. Their unique association with symbiotic bacteria (*Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae) make them more effective compared to chemical insecticides. Soil has been one of the most difficult environments in which to achieve biological control of insect pests. These nematodes are adapted to soil and have been especially effective as inundative biological control agents against a number of soil insect pests (Klein, 1990). They are also effective against a number of insect pests that occur in cryptic habitats (*e.g.* tree boring insects). Major EPN species being used for biological control presently are *Steinernema carpocapsae, S. feltiae, S. riobrave, S. scapterisci, Heterorhabditis bacteriophora, H. megidis* (Rabindra and Hussaini, 2003)

The use of EPN as biological control agents is challenging, and application techniques are still under development (Piggot and Wardlow, 2002). Effectiveness depends on the targeted host, and environmental conditions such as temperature and relative humidity (Hara *et al.*, 1993) as well as application technology, because EPN are susceptible to desiccation, temperature extremes, and ultraviolet radiation (Mason and Wright, 1997).

Despite logistical issues, the use of EPN has been successful in field and greenhouse environments to manage certain insect pests, including the black vine weevil *[Otiorhynchus sulcatus* (Coleoptera: Curculionidae)], cranberry girdler *[Chrysoteuchia topiaria*(Lepidoptera: Pyralidae)], mint root borer *[Fumibotys fumalis* (Lepidoptera: Pyralidae)], citrus weevil *[Pachnaeus litus* (Coleoptera: Curculionidae)], mole crickets *[Scapteriscus* spp. (Orthoptera: Gryllotalpidae)], billbugs *[Sphenophorus* spp. (Coleoptera: Curculionidae)], white grubs (Coleoptera: Scarabaeidae), fungus gnats [*Bradysia* spp. (Diptera: Sciaridae)] western flower thrips *[Frankliniella occidentalis* (Thysanoptera: Thripidae)], and serpentine leafminer *[Liriomyza trifolii* (Diptera: Agromyzidae)] (Hara *et al.*, 1993).

2.1. Attributes of EPNs

The EPNs have very impressive attributes as biological control agents at par with any other bioagents in use. These include, ability to kill hosts within short periods, they have a broad host range, are safe to vertebrates, plants and other non-target organisms, have no known negative effect on the environment, are easy to mass produce *in vivo* and *in vitro*, are easily applied using standard spray equipment, have the potential to recycle in the environment, high virulence, are compatible with many chemical and other biological pesticides, and wider genetic diversity, presence of chemoreceptors, are amenable to genetic selection for desirable traits, and are exempt from registration in many countries (Kaya and Gaugler, 1993). There is no need for personal protective equipment and re-entry restrictions. Insect resistance problems are unlikely. Negative attributes include their broad host range (although no negative effects on non-target hosts have been observed, this broad host range

may include some beneficial insects), narrow tolerance to environmental conditions (*e.g.* moisture requirement), poor long-term storage, poor field persistence, and relatively high cost in comparison to chemical pesticides (Kaya, 1993).

2.2. Pathogenicity, Biology and Life Cycle of EPN

Entomopathogenic nematodes are obligate, soil dwelling rhabditids, capable of infecting broad range of insect pests. The only stage that can survive outside the host is the non-feeding third infective juvenile (IJs) stage. The IJs has adapted to survive in harsh soil environment, locate and infect suitable host through natural openings (mouth, spiracles and anus) or in some species through intersegmental membranes of the cuticle before developing into adult male, female or hermaphrodite. If the mode of entry is by mouth or anus, the nematode penetrates the gut wall to reach the hemocoel, and if by spiracles, it penetrates the tracheal wall. When the infective juvenile reaches the hemocoel of a host, it releases the bacteria, which multiply rapidly in the hemolymph. The infective juveniles are closely associated with their symbiotic bacterium, which kills their host within 1-4 days after infection and protect the cadaver from colonization by other microorganisms (Thomas and Poinar, 1979). Even though the bacterium is primarily responsible for the mortality of most insect hosts, the nematode also produces a toxin that is lethal to the insect. Inside host, infective juvenile becomes feeding third stage juveniles, feeds on *Xenorhabdus* for steinernematids, *Photorhabdus* for heterorhabditids bacteria and liquefying host tissue and molts to the fourth stage prior to maturing into a adults males and females of the first generation. After mating, the females lay eggs that hatch as first-stage juveniles that molt successively to second, third, and fourth-stage juveniles and then to males and females of the second generation. The adults mate and the eggs produced by these second-generation females hatch as first-stage juveniles that molt to the second stage. The late second-stage juveniles cease feeding, incorporate a pellet of bacteria in the bacterial chamber, and molt to the third stage (infective juvenile), retaining the cuticle of the second stage as a sheath, and leave the cadaver in search of new hosts as nutritive conditions are depleted in dead cadavers (Nguyen and Smart, 1992; Wouts, 1980; Johnigk and Ehlers, 1999). Steinernematid infective juveniles may become males or females, where as heterorhabditids develop all juveniles into self-fertilizing hermaphrodites in first generation. In the second generation, males, females, and hermaphrodites are produced. Thus, steinernematids require a male and a female infective juvenile to invade an insect host to produce progeny, whereas heterorhabditids need only one infective juvenile to penetrate into a host as the resulting hermaphroditic adult is self-fertile. The life cycle is completed in a 6 to 8 days in steiernematids and 12 to 14 days in heterorhaditids at 25°C in *Galleria mellonella* (Kaya and Koppenhofer, 1999). The nematode/bacterium association is highly specific. In the infective juvenile, the bacterial cells are housed in a vesicle in the anterior part of the intestine for steinernematids and in the intestinal tract for heterorhabditids.

The insect cadaver becomes red if the insects are killed by heterorhabditids and cream if killed by steinernematids (Kaya and Gaugler, 1993). The color of the host body is indicative of the pigments produced by the monoculture of mutualistic bacteria growing in the hosts. Nematode growth and reproduction depend upon

conditions established in the host cadaver by the bacterium. The bacterium further contributes anti-immune proteins to assist the nematode in overcoming host defenses, and anti-microbials that suppress colonization of the cadaver by competing secondary invaders. Conversely, the bacterium lacks invasive powers and is dependent upon the nematode to locate and penetrate suitable hosts.

2.3. EPN Bacterium Complex

Xenorhabdus and *Photorhabdus* are motile, Gram negative, facultative, non-spore forming, anaerobic rods in the family Enterobacteriaceae. Major differences occur between the 2 bacterial genera (Boemare, 2002). For example, most *Photorhabdus* spp. are luminescent and catalase positive, whereas *Xenorhabdus* spp. have no luminescence and are catalase negative. Both bacterial genera produce phenotypic variant cell types called primary form (phase I) and secondary form (phase II) (Forst and Clarke, 2002). The primary form is the cell type naturally associated with the nematodes, whereas the secondary form can arise spontaneously when the bacterial cultures are in the stationary non-growth stage. The *Xenorhabdus* secondary form can revert to the primary form, but this phenomenon has not been documented for *Photorhabdus* spp. Differences between the primary and secondary forms occur. For instance, the primary form produces antibiotics, adsorbs certain dyes, and develops large intracellular inclusions composed of crystal proteins, whereas the secondary form does not or only weakly produces antibiotics, does not adsorb dyes, and produces intracellular inclusions inefficiently. The primary form is superior to the secondary form in its ability to support nematode propagation in vitro, although some evidence suggests that this is not always the case (Volgyi, 1998). The reason for the occurrence of the 2 forms is not known. The relationship between the nematode and bacterium is truly mutualistic for the following reasons: the nematode is dependent upon the bacterium for (1) quickly killing its insect host, (2) creating a suitable environment for its development by producing antibiotics that suppress competing microorganisms, (3) transforming the host tissues into a food source, and (4) serving as a food resource. The bacterium needs the nematode for (1) protection from the external environment, (2) penetration into the host's hemocoel, and (3) inhibition of the host's antibacterial proteins. Without the nematode the bacteria cannot survive well in the natural environment and are generally not pathogenic when ingested by a host (Akhurst, 1982; Akhurst and Boemare, 1990).

Entomopathogenic nematodes and their endosymbiotic bacteria are potent bioinsecticides that can control a wide variety of economically important agricultural pests (Shapiro - Ilan *et al.*, 2002). Due to their sensitivity to ultraviolet light and desiccation (Georgis and Gaugler, 1991), entomopathogenic nematodes have been most successful at suppressing populations of ground-dwelling pests or pests in other protected environments (*e.g.*, greenhouses). Successful pest control with nematodes requires a proper match of the nematode to the host species and favorable economics relative to the value of the commodity and the cost of competing pest control strategies. To be effective, entomopathogenic nematodes must generally be applied at rates of 2.5×10^9/ha or higher (Georgis and Hague, 1991; Georgis *et al.*, 1995). Some of the pests that have been targeted commercially with entomopathogenic nematodes are listed in Table 12.2. In addition to controlling harmful insect pests,

new frontiers are opening by using entomopathogenic nematodes, and more so, their symbiotic bacteria or associated metabolites to suppress plant parasitic nematodes (Gouge *et al.*, 1994) and as antimicrobial agents in pesticide and pharmaceutical applications. Furthermore, toxins produced by the bacteria are being investigated for their suitability as alternatives to other orally active insecticides such as toxins produced by *Bacillus thuringiensis* (Bowen *et al.*, 1999).

Table 12.2: Commercial Use of EPNs, *Steinernema* and *Heterorhabditis* as Bio-insecticides

EPN Species	*Major Pest(s) Targeted - as Recommended by various Commercial Companies*
Steinernema glaseri	White grubs (scarabs, especially Japanese beetle, *Popillia* sp.
Steinernema kraussei	Black vine weevil, *Otiorhynchus sulcatus*)
Steinernema carpocapsae	Turfgrass pests- billbugs, cutworms, armyworms, sod webworms, chinch bugs. Orchard, ornamental and vegetable pests - codling moth, cranberry girdler, dogwood borer and other clearwing borer species, black vine weevil, peachtree borer, shore flies (*Scatella* spp.)
Steinernema feltiae	Fungus gnats (*Bradysia* spp.), shore flies, western flower thrips
Steinernema scapterisci	Mole crickets (*Scapteriscus* spp.)
Steinernema riobrave	Citrus root weevils (*Diaprepes* spp.)
Heterorhabditis bacteriophora	White grubs (scarabs), cutworms, black vine weevil, flea beetles, corn root worm
Heterorhabditis megidis	Weevils
Heterorhabditis indica	Fungus gnats, root mealybug, grubs
Heterorhabditis marelatus	White grubs (scarabs), cutworms, black vine weevil

2.3. Searching Behaviour

Entomopathogenic nematodes use two search strategies: ambushers or cruisers (Grewal *et al.*, 1994a). Ambushers such as *S. carpocapsae* have an energy-conserving approach and lie-in-wait to attack mobile insects (nictitating) in the upper soil by direct contact (Campbell *et al.*, 1996). Cruisers like *S. glaseri* and *H. bacteriophora* are highly active and generally subterranean, moving significant distances using volatile cues and other methods to find their host underground. Some nematode species such as *S. feltiae* and *S. riobrave* use an intermediate foraging strategy (combination of ambush and cruiser type) to find their host.

2.4. Recycling of Nematodes

Recycling is desirable after an application of entomopathogenic nematodes because it can provide additional and prolonged control of a pest. The abiotic and biotic factors that affect persistence, infectivity, and motility of infective juveniles influence nematode recycling. Because they are obligate pathogens, the availability of suitable hosts is a key to recycling of the nematodes. Recycling is rather common (Klein, 1993) after nematode application but is probably not sufficient for prolonged host suppression, and the nematodes have to be re-applied to maintain adequate control of soil insect pests.

2.5. Dispersal of Juveniles

The juveniles of steinernematids and heterorhabditids disperse vertically and horizontally, both actively and passively (Epsky *et al.*, 1988; Parkman *et al.*, 1993). Passively, they may be dispersed by rain, wind, soil, humans, or insects. Active dispersal may be measured in centimetres, while passive dispersal by insects may be measured in kilometres (Smart and Nguyen, 1994).

2.6. Survival of Juveniles

In general, entomopathogenic nematodes do not have a long shelf life. Many microbial insecticides, including *Bacillus thuringiensis*, have a resting stage facilitating longterm storage. The infective juveniles do not feed but can live for weeks on stored reserves as active juveniles, and for months by entering a near-anhydrobiotic state. This is almost certainly the most important survival strategy for the nematode. The length of time that juveniles survive in the soil in the absence of a host depends upon such factors as temperature, humidity, natural enemies, and soil type. Generally, survival is measured in weeks to months, and is better in a sandy soil or sandy-loam soil at low moisture and with temperatures from about 15-25° C than in clay soils and lower or higher temperatures (Ames, 1990; Kaya, 1990; Kung, 1991). Extended exposure to temperature extremes (below 0°C or above 40°C) is lethal to most species of entomopathogenic nematodes. In the soil environment, infective juveniles are normally buffered from temperature extremes. For storage, the best longevity of infective juveniles is between 5 and 15°C. At higher temperatures, the infective juveniles have increased metabolic activity and deplete their energy reserves, shortening their life span (Brown and Gaugler, 1996).

UV can kill nematodes within minutes. Direct exposure to UV light (*i.e.* sunlight) can be minimized by applying infective juveniles early in the morning or evening, or using sufficient amounts of water to wash the infective juveniles into the soil. Infective juveniles can survive low moisture conditions by lowering their rate of metabolism. Gradual water removal from the infective juveniles gives them time to adapt to the desiccating conditions (Patel *et al.*, 1997; Solomon *et al.*, 1999).

Soil texture affects infective juvenile survival, with the poorest occurring in clay soils. The poor survival rate in clay soils is probably due to the lower oxygen levels in the smaller soil pores. Oxygen is also a limiting factor in water-saturated soils and soils with high organic matter content, but pH does not have a strong effect on infective juvenile survival.

3. Mass Production and Formulation of EPN

3.1. Mass Production

A key factor in the success of entomopathogenic nematodes as biopesticides is their amenability to mass production. These nematodes were first cultured more than 70 years ago (Glaser, 1940), and currently they are commercially produced using three culture methods: *in vivo* and *in vitro* solid and liquid culture (Friedman, 1990). Each approach has advantages and disadvantages relative to cost of production, capital outlay, technical expertise required, economy of scale, and product quality,

and each approach has the potential to be improved. A variety of formulation options are available (Georgis *et al.*, 1995)

Entomopathogenic nematodes are easily cultured either *in vivo* or *in vitro* for laboratory tests or for commercial production (Friedman, 1990). *In vivo* culture is a two-dimensional system that relies on production in trays and shelves. The wax worm, *G. mellonella*, is the insect of choice for *in vivo* production because it is produced commercially in large numbers and well defined diet material is available. *In vivo* production is labor intensive, lacks economies of scale, and is costly, but it is also simple and reliable and results in high quality nematodes (Shapiro-Ilan, 2003). A system based on the White trap (White, 1927), which takes advantage of the infective juvenile's natural migration away from the host cadaver upon emergence is widely used. The methods described consist of inoculation, harvest, concentration, and (if necessary) decontamination. Insects are inoculated with nematodes on a dish or tray lined with absorbent paper (*e.g.*, filter paper) or another substrate conducive to nematode infection such as soil or plaster of Paris. After 2–5 days, infected insects are transferred to the White traps; if infections are allowed to progress too long before transfer, harm to nematode reproductive stages may occur, and the cadavers will be more likely to rupture (Shapiro – Ilan, 2001). White traps consist of a dish on which the cadavers rest surrounded by water, which is contained by a larger dish or tray. The central dish (containing the cadavers) provides a moist substrate for the nematodes to move upon, *e.g.*, an inverted petri dish lid lined with filter paper or filled with plaster of Paris. The progeny infective juveniles that emerge migrate to the surrounding water where they are trapped and subsequently harvested. The choice of host species and nematode for *in vivo* production should ultimately rest on nematode yield per cost of insect and the suitability of the nematode for the pest target. Nematode quality appears to be greater when cultured in hosts that are within the nematode's natural host range (Abu Hatab and Gaugler, 2001). Furthermore, nematodes can adapt to the host they are reared on (Stuart and Gaugler, 1996), which could reduce field efficacy if that host is not related to the target. Therefore, although *G. mellonella* may often be the most efficient host to use, it may not be the most appropriate "medium" for maximizing efficacy versus a particular target pest.

There are only a couple of entomopathogenic nematodes not amenable to culture in *G. mellonella* (due to extremes in host specificity): *Steinernema kushidai* is most amenable to culture in scarab beetle larvae (Coleoptera: Scarabaeidae) (Kaya and Stock, 1997) and *Steinernema scapterisci* is most amenable to mole crickets (*Scapteriscus* spp.) (Grewal *et al.*, 1999). Other hosts in which *in vivo* production has been studied include the navel orangeworm (*Amyelois transitella*), tobacco budworm (*Heliothis virescens*), cabbage looper (*Trichoplusia ni*), pink bollworm (*Pectinophora gossypiella*), beet armyworm (*Spodoptera exigua*), corn earworm (*Helicoverpa zea*), gypsy moth (*Lymantria dispar*), house cricket (*Acheta domesticus*) and various beetles (Coleoptera) including the yellow meal worm (*Tenebrio molitor*) (Blinova and Ivanova, 1987).

For large-scale production, *in vitro* methods using 3- dimensional solid media or liquid fermentation methods have been employed but it is not cost effective and high capital requirement is needed and the inability of the amphimictic adults to

mate under liquid culture conditions (Gaugler and Han, 2002). Yang *et al.* (1997) reported reduced quality in *S. carpocapsae* produced in solid culture compared with *in vivo* culture. Without sophisticated mechanization (*e.g.*, bulk sterilization) solid culture may not offer substantial advantages in cost efficiency relative to *in vivo* production (a cost analysis is warranted). Yet large - scale mechanization for solid culture requires substantial capital. If *in vitro* solid culture is to be adopted on wider scale, efficiency will have to be increased by finding less capital - intensive methods of mechanization.

3.2. Formulation

Regardless of culture method, once entomopathogenic nematodes are commercially produced they must be formulated for delivery and application (Georgis, 1990). An effective formulation provides a suitable shelf life, stability of product from transport to application, and ease of handling. Shelf life, in most entomopathogenic nematode formulations, is obtained by reducing nematode metabolism and immobilization, which may be accomplished through refrigeration and partial desiccation. Optimum storage temperature for formulated nematodes varies according to species: generally, steinernematids tend to store best at temperatures near 4–8°C whereas heterorhabditids have longer shelf life at temperatures close to 10–15°C. The climate of origin is predictive of the optimum storage temperature, *e.g.*, *H. indica*, a nematode originating only in warm climates, stores better at 15–20 than at 10°C (Shapiro *et al.*, 1999).

Various formulations for entomopathogenic nematodes have been reported including activated charcoal, alginate and polyacrylamide gels, baits, clay, peat, polyurethane sponge, vermiculite, and water-dispersible granules (WDG) (Georgies *et al.*, 1995). Due to cost, *in vivo* producers tend to use low-technology formulations such as sponge and paste. The nematodes are not desiccated and tend to retain high viability. However, these formulations cannot be packaged at high densities and are therefore not appropriate for large - scale usage because of labor requirements in application. Formulations used by most *in vitro* producers include clay, gels, vermiculite, and WDG. For example, a successful non-desiccated formulation has been developed for *in vitro* produced nematodes based on vermiculite, which allows a shelf life of at least one month for *H. megidis* and 2–3 months for steinernematids.

4. Plantations Insect Pests are Promising Targets–Why ?

Plantations in India are major commercial crops grown varying from tropical evergreen Western ghats regions of Kerala, Karnataka and Tamil Nadu to dry regions of Andhra Pradesh, Maharasthra and Odisha. They are one of the higher foreign exchange earners besides contributing neutraceuticals supply. Insects, which are an integral part of the existing ecosystem are also the major biological determinants influencing the development and destruction of plantation crops *viz.*, seedlings damage in nurseries, young plants at establishment stage in main field, mature old plantation destruction, seeds in stand and storage. As against agriculture, which is man-made practice in the limited and controlled area, plantations some time cover the thick forests, incessant continuous rains and unapproachable areas, imposing

serious restrictions on control operations. Biological control programmes for the management of insect pests in such areas with entomopathogenic nematodes holds good promise along with judicious use of chemicals, wherever feasible. In India, a number of insect pests belonging to the coleoptera, lepidoptera, thysonoptera, hemiptera *etc.* are serious pests of plantations from seedling stage in plantations nursery to the standing tree in plantation gardens, as root feeders, defoliators, stem borers or soil inhibiting borne pests. Some of these are difficult to manage by virtue of their long life cycle, sometimes extending to one to two year and peculiar feeding habit in hidden tunnels or galleries, which are otherwise, difficult to manage by any other known method of insect pest management.

In line with all the above, the EPNs, by virtue of the presence in the local environment, if explored and proved promising against the plantation insect pests, could become an important component of IPM programme against plantation insect pests. The strongest benefit is the ability of self propagation and establishment for many year in the ecosystem, offering continuation of population at the site of release Hussaini *et al.* (2003) have discussed that the characterization of traits related to the control of potential of species (strains) of EPN is the key for a successful biological pest control with insecticidal nematodes. This has to be done with wide range of insect pests including horticulture, agriculture and plantation importance, which has been missing till date in India.

5. Current Use of EPN as Bio-agents in IPM of Plantation Crops in India

As EPN are compatible with many control measures, numerous opportunities exist for including these successfully in IPM programmes with minimal reliance on chemical pesticides, and involving more and more of other natural enemies and pathogens.

In India work on steinernematids started in the nineteen sixties. Use of DD-136 for control of pests of rice, sugarcane and apple were discussed by Rao and Manjunath (1966). In the seventies Singh and Bardhan (1974) worked on mortality in laboratory and field trials, life cycle and compatibility of DD-136 with insecticides and fertilizers.

5.1. Coconut

5.1.1. Red Palm Weevil (*Rhynchophorus ferrugineus*)

In general, 5-7 per cent palms are infested by red palm weevil in the country and being a concealed borer it becomes fatal enemy of coconut on most occasions. Higher virulence of local entomopathogenic nematode (EPN) strain of *Heterorhabditis indica* (LC_{50} =355.5 IJ) in the suppression of grubs as well as greater susceptibility (82.5 per cent) of pre-pupal stage than that of grubs stage was indicated (Figure 12.1). Synergistic effect of *H. indica* (1500 IJs) with imidacloprid (0.002 per cent) in field trials was indicated. Placement of three filter paper sachets containing 12-15 *H. indica* infected *Galleria mellonella* cadavers on the leaf axils after application of 0.002 per cent imidacloprid could recover 60 per cent infested palms (Josephrajkumar *et al.*, 2013).

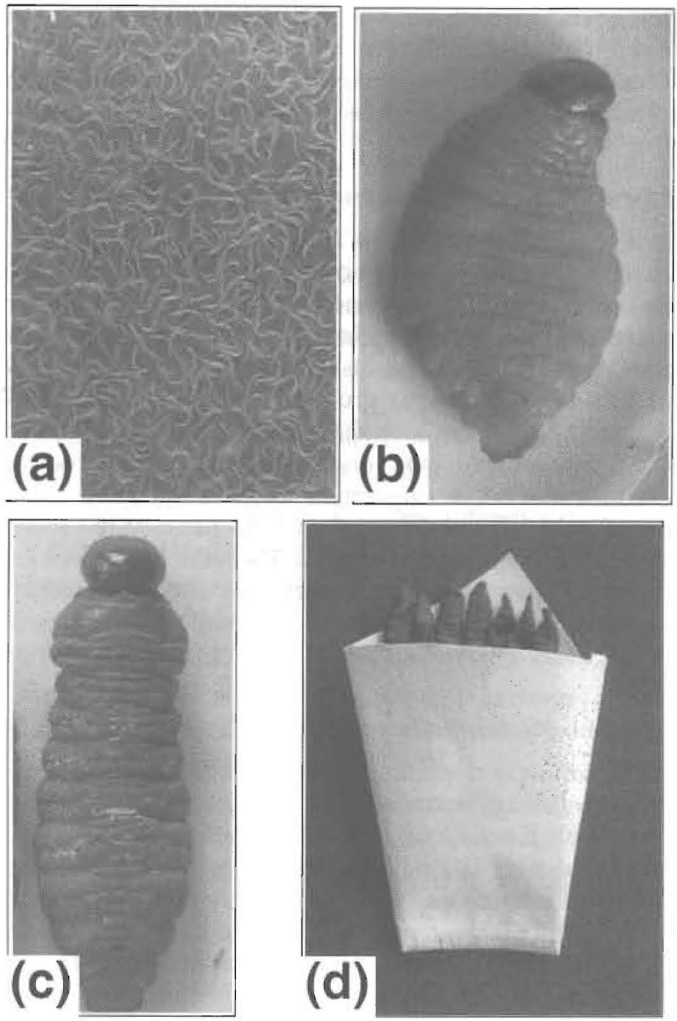

Figure 12.1

(a) Infective juveniles of EPN, (b,c) EPN infected Red palm weevil grubs and pupa, (d) EPN infected cadavers in paper boats for leaf axil placement.

Talc based local strain of *H. indica* formulation fortified with chitosan 0.25 per cent and admixed with sand were filled in leaf axils of coconut crown as prophylactic treatment against infestation of red palm weevil on a susceptible host *viz.*, Chowghat Green Dwarf (CGD). It was found that *H. indica* formulation proved efficacious to the tune of 94.4 per cent in preventive treatment while 33.3 per cent untreated control palms (CGD) succumbed to red palm weevil infestation (CPCRI, 2010).

5.1.2. Rhinoceros Beetle (*Oryctes rhinoceros*)

The entomopathogenic nematode, *Steinernema abbasi* was pathogenic to third instar grubs of rhinoceros beetle at 350 IJS/cc of vermicompost in laboratory

bioassay. *Steinernema carpocapsae* was oriented to over 7.3 cm in 72 hours of inoculation using volatile cues in vermicompost to find *O. rhinoceros* grubs. EPN, *S. carpocapsae* infected *G. mellonella* cadaver @ one/500 cm^3 was found effective in the bio-management of rhinoceros grubs (neonates) in vermicompost (CPCRI, 2014; Jagadish Patil *et al.*, 2012).

5.1.3. Root Grub (*Leuchopholis coneophora*)

S. carpocapsae (900 IJ and 1200 IJ) admixed with imidacloprid (0.250, 0.125, 0.125, 0.063, 0.031, 0.015 and 0.008 per cent) and exposed to white grub indicated a significantly higher mortality in all nematode-imidacloprid combinations after 7 days. The interaction between imidacloprid and nematodes was found to be synergistic in all combinations (Jagadish Patil *et al.*, 2013). The EPN, *Steinernema carpocapsae* at 8000 to 16000 IJs per grub in combination of 1 to 0.0001 per cent imidacloprid caused 72 per cent mortality in root grub compared to challenging of grubs with EPN alone under *in vitro* condition. Two rounds of root zone drenching of liquid EPN formulation, *S. carpocapsae* @$0.5x10^6$ IJs $palm^{-1}$ during June-July and September-October as on trial resulted in 61 per cent reduction of root grub population in costal sandy soils of Kasaragod. The reduction of root grub population increased with increase in nematode density per palm and number of treatments (CPCRI, 2014).

5.2. Arecanut

5.2.1. Root Grub (*Leuchopholis* sp.)

One round root zone drenching of liquid EPN formulation, *S. carpocapsae* @$0.5x10^7$ IJs $palm^{-1}$ during September-October as on trial resulted in 41 per cent reduction of root grub population in red soils of Sringeri in Chikkamangaluru. The reduction of root grub population increased with increase in nematode density per palm and number of treatments. Nematodes in combination of imidacloprid 17.8 SL (0.004 per cent), 1ml $5L^{-1}$ water $palm^{-1}$ was found synergistic and reduced root grub population to the tune of 60 per cent. The nematode establishment was found in all treated plots (Rajkumar *et al.*, 2014)

5.3. Coffee

5.3.1. Coffee Berry Borer (CBB), *Hypothenemus hampei*

Potential of *S. carpocapsae* as a control for CBB in fallen coffee berries in Hawaii coffee fields was accomplished. All life stages of CBB are being killed by *S. carpocapsae*, with highest mortality in larvae (Jessical Manton *et al.*, 2012).

5.4. Cardamom

5.4.1. Root Grub [*Basilepta fulvicorne* (Jacoby)]

Root grub is a serious pest damaging the roots of cardamom. Nutrient uptake is reduced due to root damage leading to yellowing of leaves; the pest problem is severe in less shaded area. Beetles occur in March-April and August-September. Females lay about 124-393 eggs in batches of 12-63 on dry cardamom leaves or

mulches. The minute creamy white grubs hatch out from eggs, fall on the ground, reach root zone and start feeding the roots. Grubs have two periods of occurrence, the first during April-July and the second during September to January. Grubs (larvae) feed on roots, become mature in 45-60 days (Varadarasan *et al.*, 2009). Soil application of *Metarhizium anisopliae* (@ 108 spores/gm) 25gms/plant mixed with compost. Native strain of EPN (*Heterorhabditis indica*) application @ 1,00,000 nematodes (IJs/plant) against early stage grubs during April/May and September/October.

On account of its versatality, persistence and specificity EPN is all likely to become an essential component of IPM strategies in sustainable pest mangment of plantation crops, as organic farming is widely promoted in this part of the country.

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