Biosuppression of Coconut Pests with Entomopathogenic Nematodes

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Entomopathogenic nematodes (EPNs) as a bio-control agent have proven successful and are now commercially mass produced in six of the seven continents to treat pest problem in agriculture, horticulture and forest crops. They actively search for the host, including those in cryptic habitat stages of insect pests in soil environment. They can be considered as good candidates for integrated pest management in sustainable agriculture due to plants and mammals which are not adversely affected and have ease of mass production. We present a general overview on the current state of knowledge of EPN and their mutualistically associated bacteria and their utilization as biological control agent against insect pests in world and India are briefly presented in this chapter.

Key words: Entomopathogenic nematodes, steinernematids, heterorhabditids, mass production and coconut

The application of insecticides has been the control method commonly used, but its rejection by society is increasing, due to effects on non-target organisms, water contamination, residues found in fruit and vegetables and development of resistance among insect pests. In this context, biological control is becoming a useful alternative, as part of an integrated pest management (IPM) strategy. Entomopathogenic nematodes (EPNs) play an important role as biocontrollers additionally, EPNs focus on the soil stages, which are difficult to control with chemical pesticides.

The interest in the use of entomopathogenic nematodes as biological pest control agents has increased exponentially over the past decades. A hundred different laboratories explore these nematodes and their bacterial symbionts in more than 60 countries from every inhabited continent. Despite research breadth that extends from molecular biology to field ecology, the discipline is unified by common interest in biological control. Thirty years ago, the idea of using nematodes to control pest populations was vague promise held by the 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. The entomopathogenic nematodes have proven particularly successful and are now commercially mass-produced in six of the seven continents to treat pest problems in agriculture, horticulture and human husbandry. The ease of mass production and exemption from registration requirements are the two major reasons for early interest in the commercialization of entomopathogenic nematodes. However, demonstrations of practical use, particularly in Europe and North America and subsequently in Japan, China and Australia, spurred developments across the world that have led to the availability of nematodes against pests that were once thought impossible to control. Entomopathogenic nematodes (EPNs) of the families, Steinernematidae and Heterorhabditidae are known to be lethal pathogens of insect pests. These pathogens contribute to the regulation of natural

populations of insects, but the main interest in them is an inundatively applied biocontrol agent (Kaya and Gaugler, 1993). Their success in this role can be attributed to the unique partnership between a host-seeking nematode and a lethal insect pathogenic bacterium. Because of their biocontrol potential, considerable attention has been directed over the past few decades to genus, Heterorhabditis and Steinernema and their respectative bacterial partners, Photorhabdus and Xenorhabdus (Forst and Clarke, 2002).

Entomopathogenic nematodes

Entomopathogenic nematodes (genera Steinernema and Heterorhabditis) are biocontrol agents used to target a variety of economically important insect pests (Grewal et al., 2005), kill insects with the aid of mutualistic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Poinar, 1990; Lewis and Clarke, 2012), which have been described from 23 nematode families (Koppenhofer 2007). So far more than 100 species in families Steinernematidae and Heterorhabditidae have been described from all continents except Antarctica and this number is growing every year (Nguyen & Hunt, 2007). A major difference between steinernematids and heterorhabditids is that all but one species in the former group are amphimictic, whereas species in the latter group are hermaphrodites in the first generation but amphimictic in the following generation. 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. These nematodes are unique because, they are the only nematodes which have evolved the ability to carry and introduce the symbiotic bacteria into the body cavity of the insect, they are the only organisms with a wide host range of insects, they can be cultured on a large scale either on hosts or on artificial media, can be easily applied in the field with standard spray equipment and can actively find and penetrate the susceptible host and cause up to 100% mortality within few days. Since their discovery in 1927, EPN have been considered as valuable alternatives to chemical pesticides as they can parasitize a wide range of insects that are agricultural pests and insects in cryptic habitats in many parts of the world (Table 1).

Table 1. 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

Because of these attributes, as well as their ease mass production and exemption from registration, a number of commercial enterprises produce these nematodes as biological "insecticides" that are used to control a variety of economically important insect pests. An excellent example of a situation in which a nematode may replace chemicals for control of an insect is the black vine weevil (*Otiorhynchu ssulcatus*) in cranberries. When *Heterorhabditis bacteriophora* "NC" strain was applied, it provided 70% control of the weevils soon after treatment and was still providing the same level of control a year later, Diaprepres root weevil, *Diaprepes abbreviatus* (L.), fungus gnats (Diptera: Sciaridae), thrips (Thysanoptera), and various white grubs (Coleoptera: Scarabaeidae) (Klein, 1990; Shapiro-Ilan *et al*., 2002, 2014; Grewal *et al*., 2005).Preventative applications of the *Steinernema carpocapsae* (Weiser), can reduce peach tree borer, *Synanthedon exitiosa* infestations at the same level as chlorpyrifos at North America. Specifically, when nematodes were applied prophylactic-ally infestations were reduced by 77% to 100% (Shapiro-Ilan *et al*., 2009& 2015). In India, some of the plantation pests (Table 2) were managed by soil drenching of *S. carpocapsae* @0.5x107 IJs palm^{-1} resulted in 41 per cent reduction of white grub (*Leucopholis* spp.) population in arecanut at Sringeri, Karnataka (India). Nematodes in combination of imidacloprid 17.8 SL (0.004 per cent), 1 ml 5L^{-1} water palm^{-1} found synergistic and reduced root grub population to the tune of 60 per cent (Rajkumar *et al.,* 2014). In coconut, two round root zone drenching of liquid formulation, *S. carpocapsae* @ 0.5 x 10^6 IJs palm^{-1} for two years during June/July and September/October resulted in 61 per cent reduction of root grub (*Leucopholis coneophora*) population in costal sandy soils of Kasaragod, Kerala (India) (Rajkumar and Subaharan 2016). Soil application of native strain of *Heterorhabditis indica* application @ 1, 00,000 nematodes (IJs/plant) against early stage of cardamom root grubs (*Basilepta fulvicorne* (Jacoby)) during April/May and September/October provided significant control (Varadarasan *et al.,* 2009)and they are compatible with most pesticides. The efficacy of entomopathogenic nematode applications, however, can be limited by adverse environmental conditions such as UV radiation or desiccation, extreme temperatures (Shapiro-Ilan et al., 2006).

Table 2. 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 minor (*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*

Infective Juvenile (IJ) and mode of action

The only stage that survives outside of the host is the non feeding infective third stage juvenile of these nematodes which are found free living in soil under diverse ecological conditions and in all kinds of habitats (Hominick *et al.*, 1996). The IJ is the stage that is purchased in commercial products. These IJ also known as dauer juvenile (DJ) is more resistant than other stages to environmental conditions and survive in the soil environment for extended periods, until they find a suitable host (Lewis and Clarke, 2012; Shapiro-Ilan et al., 2014). The IJ carries cells of bacterial symbionts in its intestine. When the IJ finds susceptible insect host, enter through natural openings (mouth, anus, and spiracles) for Steinernematidae or sometimes through the cuticle for *Heterorhabditis*. After entering the insect's heomocoel, nematodes release their bacterial symbionts, which are primarily responsible for killing the host within 24 to 48 h. The bacterium produces antibiotics that prevent other microorganisms from colonizing the cadaver. In addition to serving as a food source for the nematode, the bacterium digests the host tissues, thereby providing suitable nutrients for nematode growth and development. The nematodes molt and complete two to three generations within the host cadaver, as resources of the insect are depleted and crowding occurs, IJ are produced after which IJs exit the cadaver to find new hosts to attack (Poinar, 1990; Lewis and Clarke 2012). The reproductive potential of entomopathogenic nematodes is very high. Thousands of nematodes can be produced from a single infected insect host.

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 reapplied to maintain adequate control of soil insect pests.

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 centimeters, while passive dispersal by insects may be measured in kilometers (Smart and Nguyen 1994).

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 15-25^0 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.

Mass Production and Formulation of EPN

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 *et al.,* 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. 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 (Figure 1). 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 (Figure 1) 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.) 9Grewal *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 involves high cost and high capital requirement 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.

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, steirnernematids tend to store best at temperatures near 4–8^{o}C whereas heterorhabditids have longer shelf life at temperatures close to 10–15^{o}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^{o}C (Shapiro 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 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 1 month for *H. megidis* and 2–3 months for steinernematids (GraemeGowling, MicroBio, Cambridge, UK, personal communication).

Current use of EPN as bio-agents in IPM of coconut

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

Rhinoceros beetle (*Oryctes rhinoceros*)

The entomopathogenic nematode, *Steinernema carpocapsae and S. abbasi* was pathogenic to grubs of rhinoceros beetle at 350 IJS/cc of vermicompost. *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 (Patil *et al*., 2012).

Root grub (*Leucoopholis coneophora*)

The *S. carpocapsae* (900 IJ and 1200 IJ) admixed with imidacloprid (0.250 to 0.008%) 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 (Patil *et al.,* 2013). Two round root zone drenching of aqua formulation of indigenous EPN, *S. carpocapsae* @ $0.5x10^6$ IJs $palm^{-1}$ during June-July and September-October resulted in 61% reduction of root grub population in costal sandy soils of Kerala. The reduction of root grub population increased with increase in nematode density per palm and number of years of treatments(Rajkumar and Subharan 2016).

Red palm weevil (*Rhynchophorus ferrugineus*)

In general, 5-7% palms are infested by red palm weevil in the country and being a concealed borer, it becomes fatal enemy of coconut on most occasions.

Placement of three filter paper sachets containing 12-15 *H. indica* infected *Galleria mellonella* cadavers on the leaf axils after application of 0.002% imidacloprid could recover 60% infested palms (Joseph Rajkumar *et al*., 2013).

Conclusion

Entomopathogenic nematodes are economically important bio-control agents and found effective in managing cryptic soil dwelling insects pests , thus attracted wide spread commercial interests in world and India. These EPNs have advantageous of ease of production and application, mammalian safety and exception from registration in many countries. They also possess a broad host range which are compatible with other management components. Not only these, they are widely distributed in different agro-ecosystem and can be formulated and stored for reasonable length of time. Recently, improvement in nematode formulation, application approaches and strain improvement have been made to enhance EPN application efficacy. Advanced research towards lowering the product costs, increasing shelf life and increasing product availability will stimulate the extensive use of EPNs as bio-control. With these progress in research, EPN will serve to reduce insecticide inputs going to agro-ecosystem and contribute to the stabilization of crop yield and the environment.

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