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Chapter - 2

Entomopathogenic Nematode a Potential Tool for Biological Control of Insect Pests

Saroj Yadav and Jaydeep Patil

Entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae (Rhabditida) have been used as effective biological control agents against a wide spectrum of insect pests. Steinernematids are symbiotically associated with entomopathogenic bacteria (EPBs) from the genus *Xenorhabdus,* and heterorhabditid nematodes are symbiotically associated with EPBs from the genus *Photorhabdus.* The bacterial symbionts produce wide range of toxins, hydrolytic exoenzymes, and antibacterial compounds. These compounds not only kill and bioconvert infected larvae, but also preserve the cadavers from being consumed by other soil organisms. There have been recent advances in the technology of mass producing and formulating nematodes. These recent advances, together with the need to reduce pesticide use, have resulted in a surge of scientific and commercial interest in EPNs and their symbiotic bacteria. Many species and strains of potential control organisms have to be evaluated to elaborate a new biological control technique.

Keywords: Biological control agents, entomopathogenic nematodes, mass production and formulations etc.

Introduction

Nematodes are non-segmented, elongated roundworms that are colorless, without appendages, and usually microscopic. There are non-beneficial and beneficial nematodes. Non-beneficial nematodes cause damage to crops and other types of plants are also called “plant parasitic nematodes”. Beneficial nematodes attack soil-borne insect pests, yet are not harmful to humans, animals, plants, or earthworms, and can therefore be used as biological control organisms (Denno *et al.,* 2008). Beneficial nematodes cause disease in insect are referred to as “entomopathogenic” and have the ability to kill insects.

Indiscriminate use of chemical pesticides for the management of insect pests in different agro ecosystems has been raised many environmental concerns *viz.* ground water contamination, residue in food, resistance development, soil pollution, air pollution, secondary pest outbreak, pest resurgence, etc. (Zimmerman and Cranshaw 1990). As a substitute to pesticides, biological control agents like entomopathogenic fungi, bacteria, viruses and nematodes have gained more importance due to its ecofriendly properties. Biopesticides have been accepted as important component of Integrated Pest Management. Selected species of fungi, bacteria, viruses and nematodes with established insecticidal activities constitute biocontrol agents which have been formulated into biopesticides for the management of insect pests. So far, 3,000 microbial species have been identified to cause diseases in insects (Dhaliwal *et al.* 2013).

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are obligate parasites of insects (Poinar, 1990; Lewis and Clarke, 2012). Nematodes have a symbiotic relationship with a bacterium (*Xenorhabdus* spp. are associated with steinernematids and *Photorhabdus* spp. are associated with heterorhabditids) (Poinar, 1990). Infective juveniles nematodes (IJs), the only free-living stage, enter hosts through natural openings, or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria and the nematodes molt and complete up to three generations within the host, after which IJs exit the cadaver to search out new hosts (Kaya and Gaugler, 1993). Entomopathogenic nematodes are effective at controlling a variety of economically important pests. Entomopathogenic nematodes are currently produced by different methods either *in vivo* or *in vitro* (solid and liquid culture) (Friedman, 1990).

The keys to success with entomopathogenic nematodes are (1) understanding their life cycles and functions; (2) matching the correct nematode species with the pest species; (3) applying them during appropriate environmental conditions (soil temperature, soil moisture, sunlight); and (5) applying them only with compatible pesticides. Because entomopathogenic nematodes are living organisms, they require careful handling to survive shipment and storage as well as appropriate environmental conditions to survive in the soil after application.

Entomopathogenic Nematodes Biology

The parasitic cycle of nematodes is initiated by the third stage infective juveniles. These non-feeding juveniles locate and invade suitable host insects through natural body openings (*i.e.* anus, mouth, and spiracles) or even through the cuticle when the genus *Heterorhabditis* is concerned. Once inside the host, infective juveniles invade the hemocoel and release a symbiotic bacterium, which is held in the nematode's intestine (Poinar 1990). The bacteria cause a septicemia, killing the host within 24-48h. The infective juveniles feed on the rapidly multiplying bacteria and disintegrated host tissues. About 2-3 generations of the nematode are completed within the host cadaver. When food reserves are depleted, nematode reproduction ceases and the offspring develop into resistant infective juveniles which disperse from the dead host, and are able to survive in the environment and to seek out new hosts.

Host Range

The nematode-bacterium complex kills insects so rapidly that the nematodes do not form the intimate, highly adapted, host-parasite relationship characteristic of other insect nematode associations, e.g., mermithids. This rapid mortality permits the nematodes to exploit a range of hosts that spans nearly all insect orders, a spectrum of activity well beyond that of any other microbial control agent. In laboratory tests, *S. carpocapsae* alone infected more than 250 species of insects from over 75 families in 11 orders (Poinar 1975). The nematodes attack a far wider spectrum of insects in the laboratory where host contact is assured, environmental conditions are optimal, and no ecological or behavioral barriers to infection exist (Kaya & Gaugler 1993, Gaugler *et al.* 1997). For example, foliage feeding lepidopteran larvae are highly susceptible to infection in Petri dishes, but are seldom impacted in the field, where nematodes tend to be quickly inactivated by the environmental extremes (*i.e.,* desiccation, UV radiation, temperature) characteristic of exposed foliage. Behavioral barriers also restrict nematode efficacy to a few selected hosts or host groups (Gaugler *et al.* 1997).

Selection of Entomopathogenic Nematodes

Selection of an EPN for control of a particular pest insect is based on several factors that include the nematode's host range, host finding or foraging strategy, tolerance of environmental factors and their effects on survival and efficacy (temperature, moisture, soil type, exposure to ultraviolet light, salinity and organic content of soil, means of application, agrochemicals, and others). The four most critical factors are moisture, temperature, pathogenicity for the targeted insect, and foraging strategy (Kaya and Gaugler, 1993).

Compatibility with Other Agents and Agrochemicals

The combination of EPNs and other control agents has proved to be synergistic and produces higher mortality than either agent alone. For example, Koppenhofer and Kaya (1997) showed additive and synergistic interaction between EPNs and *Bacillus thuringiensis* for scarab grub control.

Entomopathogenic nematodes are often applied to sites and ecosystems that routinely receive other inputs that may interact with nematodes including chemical pesticides, surfactants (e.g., wetting agents), fertilizers, and soil amendments. Often it is desirable to tank mix one or more inputs to save time and money. Infective juveniles are tolerant of short exposures (2-6h) to most agrochemicals including herbicides, fungicides, acaricides, and insecticides (Rovesti and Deseo, 1990; Ishibashi 1993), and therefore, can often be tank-mixed. However, some pesticides can reduce nematode infectivity and survival (Grewal *et al*. 1998). Due to the continuous introduction of new active ingredients and formulations in different market segments and to differences in susceptibility of nematode species to pesticide formulations, it is difficult to provide up-to-date information. However, heterorhabditids tend to be more sensitive to physical challenges, including pesticides, than steinernematids.

Host Finding Mechanism of Entomopathogenic Nematodes

Host-finding strategies of entomopathogenic nematode will help you properly match nematode species to pest insects to ensure infection and control (Gaugler 1999). Only infective juvenile stage of entomopathogenic nematodes will survive in the soil and find and penetrate insect pests. Infective juvenile locate their hosts in soil by means of two strategies-ambushing and cruising (Gaugler *et al.* 1989). Ambusher species include *Steinernema carpocapsae* and *S. scapterisici*; cruisers include *Heterorhabditis bacteriophora* and *S. glaseri*. *S. riobrave* and *S. feltiae* do a bit of both ambushing and cruising (Campbell and Gaugler 1997).

Ambushing: Entomopathogenic nematodes that use the ambushing strategy tend to remain stationary at or near the soil surface and locate host insects by direct contact (Campbell *et al.* 1996). An ambusher searches by standing on its tail so that most of its body is in the air, referred to as "nictation". The nictating nematode attaches to and attacks passing insect hosts. Ambusher entomopathogenic nematodes most effectively control insect pests that are highly mobile at the soil surface, such as cutworms, armyworms, and mole crickets.

Cruising: Entomopathogenic nematodes that use the cruising strategy are highly mobile and able to move throughout the soil profile. Cruisers locate their host by sensing carbon dioxide or other volatiles released by the host. Cruiser entomopathogenic nematodes are most effective against sedentary and slow-moving insect pests at various soil depths, such as white grubs and root weevils.

Mass Production

Entomopathogenic nematodes can be mass-produced by *in-vivo* or *in-vitro* methods. The wax moth, *G. mellonella* larvae are most commonly used to rear nematodes because of their commercial availability. Using the *in-vivo* process, yields between $0.5x10^5$-$4x10^5$ infective juveniles per larva, depending on the nematode species, have been obtained. During the past few years a distinct cottage industry has emerged, which utilizes the *in-vivo* process for nematode mass production for sale, especially in the home lawn and garden markets. The *in-vivo* process, however, lacks any economy of scale; the labor, equipment, and material (insect) costs increase as a linear function of production capacity. Perhaps even more important is the lack of improved quality while increasing scale. The *in-vivo* nematode production is increasingly sensitive to biological variations and catastrophes as scale increases (Friedman 1990).

First time developed the artificial culture (*in-vitro*) methods for entomopathogenic nematodes for *S. glaseri* (Glaser, 1932). The first successful commercial scale monoxenic culture was developed by Bedding and has come to be known as "solid" culture (Bedding 1981). In this method, nematodes are cultured on a crumbed polyether polyurethane sponge impregnated with emulsified beef-fat and pig's kidneys along with symbiotic bacteria. Using this method approximately $6x10^5$ - $10x10^5$ infective juveniles/g of medium were achieved (Bedding 1984). Friedman also reported the development of a liquid fermentation technique for large-scale production of nematodes (Friedman 1990). In this method, costs of production decrease rapidly up to a capacity of approximately $50x10^{12}$ infective juveniles/month. This method allows consistent production of steinernematids in as large as 80,000 litter fermenters. Recent improvements in the nematode fermentation and media formulation processes have resulted in further improvements in nematode quality and yields

Application Technology

Technology must also be developed which insure the successful application of the entomopathogenic nematode (EPN's) to the target site and also target insect, thereby increasing the probability of entomopathogenic nematode-insect interaction. Insect life-stage susceptibility is critical, since different life stages of different species are not equally susceptible. Many times, larval stages of insects such as different borers are not accessible to entomopathogenic nematodes. Evaluating the most appropriate entomopathogenic nematode strain is important for efficacy and commercial development. Nematodes' ability to effectively kill the target pest have been influenced by either abiotic factors such as soil type, soil temperature and moisture, or biotic factors, including pathogens and predators, can greatly influence the.

Application techniques, including field dosage, volume, irrigation and appropriate application methods are very important, especially if nematodes are to be integrated with other control strategies. Compatibility with a wide range of pesticides has been demonstrated. This has benefited the successful introduction with existing Integrated Pest Management programs. Crop morphology and phenology must be considered in predicting whether nematodes are viable control candidates.

Table: Efficacy of entomopathogenic nematodes against different insect pests

Crop	**Pest**	**EPN species**	**Dosage (IJs)**	**Mortality (%)**	**Reference (s)**
Apple	*Cydia pomonella* (Linnaeus)	*Steinernema carpocapsae*	5×109/ha	83	Lacey and Unruh (1998)
	Hoplocampa testudinca Klug	*S. carpocapsae*	1×105/500 cm long branch	100	Belair *et al* (1998)
Cabbage	*Plutella xylostella* (Linnaeus)	*S. carpocapsae*	75/cm2	80	Schroer and Ehlers (2005)
Citrus	*Diaprepes abbreviatus* (Linnaeus)	*S. riobrave*	Soil baiting technique	65-80	Stuart *et al* (2004)
Coffee	*Hypothenemus hampei* (Ferrari)	*Heterorhabditis* spp. *S. carpocapsae*	-	High mortality	Allard and Moore (1989), Castillo and Marban-Mendoza (1996)
Field and plantation crops	*Maladera insanabilis* Brenske	*H. bacteriophora, S. feltiae,S. glaseri*	-	Significant reduction	Bhatnagar *et al.* (2004)
Tomato	*Spodoptera litura*	*Steinernema carpocapsae*	20000 IJs/Plant.	77.5 and 75 per cent mortality	Saroj Yadav *et al* (2016)

Mushroom	*Lycoriella auripila* (Winnertz)	*S. feltiae*	3×106 /tray	91-93	Grewal *et al.* (1993)
Polyphagous pest	*Helicoverpa armigera* (Hubner)	Local isolate EPN-3 and EPN -16 (Gujarat)	2000/5 chickpea plant /pot	96.8 and 70.9	Vyas (2003), Vyas *et al* (2002)
	H. indica	90/larva	97.5		Prabhuraj *et al.* (2006)
	Holotrichia consanguinea (Blanchard)	*H. indicus*	-	Significant reduction	Singh *et al.* (2001)
	Spodoptera litura Fabricius	*H. indica, S. glaseri*	-	-	Saravanapriya and Subramanian (2007)
Potato	*Agrotis ipsilon* (Hufnagel)	*Neoaplectana sp.*	-	100	Singh (1977)
Stored product	*Plodia interpunctella* (Hubner)	*S. riobrave, S. carpocapsae, S. feltiae*	50/ individual	100	Ramos-Rodriguez *et al.* (2006)
Turf field	*Popillia japonica* Newman	*S. glaseri*	5×109/ha	44-66	Selvan *et al.* (1993)

Entomopathogenic Nematodes Formulation

Generally, the components of the formulations are: an active ingredient, a carrier and additives. Active ingredients in the formulations are EPNs, whereas the carriers used are solids, liquids, gels, and cadavers. The additives are various substances with different functions, such as absorbents, adsorbents, emulsifiers, surfactants, thickeners, humectants, dispersants, antimicrobials, and UV-ray protectors (Grewal, 2002). The main purpose of the additives used in the formulations has been to increase the survival and maintain the virulence of the EPNs.

Formulations for Storage and Transport

Aqueous Suspension: The most common EPN formulation is an aqueous suspension. It has been used mainly for storage, transportation, and application (Chen & Glazer, 2005). Storage temperatures between 4 and 15°C have produced survival times of 6–12 months for *Steinernema* spp. and 3–6 months for *Heterorhabditis* spp. (Hazir *et al.*, 2003). However, there are many factors that affect their survival time: sedimentation, high oxygen demand, decreased response of some species at low temperatures, susceptibility to microbial contamination, special storage conditions and appropriate concentration for each species (Grewal & Peters, 2005).

Synthetic Sponges: The formulation in polyurethane sponges is accomplished by applying an aqueous suspension of 500–1000 IJs cm^2, which results in an amount of 5–25 million IJs per sponge, which is subsequently placed in a plastic bag for storage. The EPNs formulated in sponges achieve a survival time of 1–3 months at 5–10°C (Grewal, 2002) and for their release, the sponges are dipped in a bowl with water.

Gels: Yukawa and Pitt (1985) described a system for nematode storage and transport. The EPNs were homogeneously mixed with absorbent materials, such as activated carbon powder, to form a cream, but this formulation has presented the drawbacks of high cost, unpleasant handling and low stability at room temperature (Grewal, 1998).

Clay and Powder: Bedding (1988) encapsulated *S. feltiae, Steinernema bibionis, Steinernema glaseri,* and *Heterorhabditis heliothidis* in a hygroscopic attapulgite clay formulation with survival time of 8 weeks at 23°C. The formulation was called a "sand wich" type, because the EPNs are stored between two layers of clay. Products with this formulation were sold, but soon were discontinued due to poor storage stability, clogging of the spray nozzles, and a low nematode-clay proportion (Grewal, 2002).

Formulations for Direct Application in the Field

Gel: With the aim of eliminating the disadvantages of releasing the EPNs from the alginate granules with sodium citrate, Kaya and Nelsen (1985) encapsulated the EPNs *S. feltiae* and *H. heliothidis* in calcium alginate granules coated by lipid membranes and fed to larvae of *Spodoptera exigua* Hübner. While feeding on the capsules, the larvae released the EPNs. When moisture was present, larval mortality was nearly 100%.

Infected Cadavers: The cadavers are another way to apply EPNs in the field (Raja *et al.*, 2015). In this formulation, the insect cadaver serves as a reservoir to store the EPNs and then they are applied in the field. Laboratory tests have indicated that this method of application produces a better distribution of the EPNs in the soil than that obtained with the aqueous solution (Shapiro & Glazer, 1996), increases infectivity (Shapiro & Lewis, 1999) and is more effective (Shapiro-Ilan *et al.,* 2003).

Table: Commercially available Entomopathogenic Nematodes

Product Name	Nematode Species	Target Pests	Producer
Ecomask	*Steinernema carpocapsae*	Caterpillars	Bio Logic
Savior Weevil larvae	*Steinernema carpocapsae*	Caterpillars	Thermo-Trilogy
Guardian	*Steinernema carpocapsae*	Caterpillars	Hydro Gardens
J-3 Max	*Steinernema carpocapsae,* *Heterorhabditis bacteriophora*	Caterpillars	The Green Spot
Heteromask	*Heterorhabditis bacteriophora*	Weevils, grubs	Bio Logic
Lawn Patrol	*Heterorhabditis bacteriophora*	Weevils, grubs	Hydro Gardens
Scanmask	*Steinernema feltiae*	Fungus Gnats	Bio Logic
Entonem	*Steinernema feltiae*	Fungus gnats	Koppert
Nemasys	*Steinernema feltiae*	Fungus gnats	E.C. Geiger

Factors Affecting Market Expansion of Entomopathogenic Nematodes

- **Markets, Crops and Target Insects:** Efficacy of entomopathogenic nematodes under the field is limited. Different product labels listed are unsuitable to the target insects and they are effect against the few selected insects and environment. Efficacy against certain insects is significantly lower than competitive products. Certain product labels recommend suboptimum application rates. Limited data on cost effectiveness in IPM programs.
- **Formulation and Shelf Life:** Refrigeration requirements and limited room temperature shelf life. Certain formulations require time for mixing and preparing spray solution. Sub-optimum storage by the distributors, dealers and growers.
- **Usage Directions:** Different product labels lack proper application directions and application requirements such as temperature, moisture, irrigation and timing. Product coverage is impractical in certain applications. Improper handling, mixing and application by end users.
- **Technical Support:** Limited experience and knowledge of farm advisors and extension personnel.
- **Cost and Gross Margins:** In general, products are more expensive than competitive products. Gross margins are generally lower for the distributors than competitive products.

Advantages of Entomopathogenic Nematode

- Broad host range of insect pest
- Rapid kill of insects
- Can actively seek or ambush host
- *In vivo* and *in vitro* mass production capability
- Application through conventional equipment
- Safety: for all vertebrates, most non-target invertebrates, and the food supply
- Little or no registration required

Disadvantages

- High cost of production
- Limited shelf-life and refrigerated storage required
- Environmental limitations: requirements for adequate moisture (to enable survival and infectivity) and temperatures (above or below that required for optimal infectivity), sensitivity to UV radiation, lethal effect of several pesticides (nematicides, fumigants and others), lethal or restrictive soil chemistries (high salinity, high or low pH, etc.).

Conclusion and Future Prospects

Entomopathogenic nematodes gain higher popularity in controlling insect pest due to the safety environmental concern. To overcome limitations in their use current efforts for improving mass-production techniques and lowering the manufacturing costs and in developing more advanced carriers and techniques in formulation to widen the IJs shelf-life. Moreover, genetic improvement may be considered as a novel venue that would help increasing nematode performance and efficacies in the field.

Further advancements are needed in the symbiotic bacteria *Photorhabdus* and *Xenorhabdus* are highly insecticidal against certain groups of insect pests, the potential of insecticidal toxins isolated from these bacteria as novel insecticidal proteins for insect control. Overall, the future use of EPNs is promising, given all the advantages they possess, as well as the increasing demand for any virulent microbial pathogen to help mitigate the environment and resistance pressure of synthetic chemical insecticides.

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