

Entomopathogenic Nematode as a Bio Insecticide

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SUMMARY

Entomopathogenic nematodes (EPNs) receiving a lot of interest because of their ability to infect and kill insects. EPNs reside naturally in the soil and are obligate parasites of a wide variety of insect species. They have evolved symbiotic relationship with insect pathogenic bacteria. Three genera of EPNs which are able to fulfil the role of vectors for entomopathogenic bacteria have been identified and reported on so far. They include species belonging to the genera *Heterorhabditis*, *Steinernema*, and *Oscheius*. Their ability to infect insects is dependent on their symbiotic association with pathogenic bacteria belonging to the genera *Photorhabdus*, *Xenorhabdus*, and *Serratia*, respectively.

INTRODUCTION

Entomopathogenic nematodes are a group of nematodes (thread worms), causing death to insects. They are multi-cellular metazoans that occupy a bio control middle ground between microbial pathogens and predator/ parasitoids, and are habitually grouped with pathogens, most likely because of their symbiotic relationship with bacteria. Entomopathogenic nematodes are specific in only infecting insects. Entomopathogenic nematodes (EPNs) live parasitically inside the infected insect host, and so they are termed as *endoparasitic*. They infect many different types of insects living in the soil like the larval forms of moths, butterflies, flies and beetles as well as adult forms of beetles, grasshoppers and crickets. EPNs have been found in all over the world and a range of ecologically diverse habitats. They are highly diverse, complex and specialized. The most commonly studied entomopathogenic nematodes are those that can be used in the biological control of harmful insects, the members of *Steinernematidae* and *Heterorhabditidae*. They are the only insect parasitic nematodes possessing an optimal balance of biological control attributes.

Nematodes as Pest Control

Using beneficial nematodes for gardening pest control has become an increasingly popular method for six reasons:

- They have an incredibly wide range of hosts and can, therefore, be utilized to control numerous insect pests.
- Entomopathogenic nematodes kill the host quickly, within 48 hours.
- Nematodes may be grown on artificial media, making a readily available and in expensive product.
- When nematodes are stored at proper temperatures (60-80 °F/15-27 °C), they will remain viable for three months and if refrigerated at 37-50 °F (16-27 °C), may last six months.
- They are tolerant of most insecticides, herbicides and fertilizers, and the juveniles can survive for a time without any nourishment while searching for an appropriate host. In a nutshell, they are resilient and durable.
- There is no insect immunity to the *Xenorhabdus* bacteria, although beneficial insects often escape being parasitized because they are more active and apt to move away from the nematode. The nematodes cannot develop in vertebrates, which make them extremely safe and environmentally friendly.

Life Cycle

Life cycle of entomopathogenic nematode includes the egg, four juvenile stages and adult. The third stage is a free-living infective juvenile (dauer stage). The infective juveniles of both *steinernematids* and *heterorhabditids* carry in its gut bacteria of the genus *Xenorhabdus* and *Photorhabdus*, respectively (Boemare *et al.*, 1993). The infective juvenile enters the host through mouth, anus or spiracles or penetrates through the intersegmental membranes of the insect cuticle as in case of *Heterorhabditis* sp. and reaches the haemocoel. In the haemocoel, infective juvenile releases cells of bacterial symbiont from its intestine. The nutrient-rich haemolymph of insect helps in the rapid multiplication of bacteria and ultimately results in killing the host within 48 h (Woodring and Kaya 1988). The infective juvenile then becomes feeding juvenile or functional third-stage juvenile and feed on the multiplying bacteria and degrading host tissues. The nematodes moult to fourth stage and finally develop into adult. The life cycle of *steinernematids* from infection to emergence of infective juveniles

ranges from 7 to 10 days and for heterorhabditids ranges from 12 to 15 days (Sundarababu and Sankaranarayanan 1998). The number of generations may be more than one within the host cadaver depending upon the available resources. Infective juveniles of Steinernematids develop into amphimictic females and males and never develop into hermaphrodites, whereas Heterorhabditids always develop into hermaphrodites in the first generation. Subsequent generation of heterorhabditids produces males, females and hermaphrodites (Dix *et al.*, 1992). First generation adults of steinernematids are termed as giant adults due to their larger size. This condition is believed to be due to the abundant available nutrition. The progeny of next generation, in most cases, find gradually depleting food supply due to regular progeny development. A full third generation progeny may be observed when the food supply is in plenty (Adams and Nguyen 2002). Juveniles developing with adequate food supply mature to adults, while those developing in crowded conditions with limited food resources results in infective juveniles. Under suitable condition infective juveniles exit the cadaver to seek new hosts.

Nematode - Bacteria Symbiosis

Infective juveniles of entomopathogenic nematode carry the bacteria *Xenorhabdus* (in case of steinernematids) or *Photorhabdus* (in case of heterorhabditids) belonging to Enterobacteriaceae (Forst *et al.*, 1997). These bacteria are Gram-negative, anaerobes, nonspore former and do not have resistant stage. Infective juveniles of *Steinernema* sp. harbour *Xenorhabdus* sp. in a special intestinal vesicle, whereas those of *Heterorhabditis* sp. carry *Photorhabdus* sp. in the anterior two third part of the intestine. Entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*, belonging to different species harbour different species of bacteria. The life cycle of nematode–bacteria association is composed of two stages: (i) a free stage in the soil, where the infective juveniles carry bacteria in their guts and search for new insect host, and (ii) a parasitic stage, where the infective juveniles infect insect, release their bacterial symbionts and reproduce in order to produce new infective juveniles. Both partners benefit from the association. The bacteria provide a nutritive medium for the growth and reproduction of nematodes. These bacteria are also useful in other two ways: (i) largely responsible for the rapid death of the host, as well as (ii) suppressing other competing organisms by the production of antibiotics. On the other hand, nematode protects the bacteria from the external environment, carries them into the insect haemocoel and in some cases inhibits the insect immune response.

Foraging Strategies

The foraging strategies of entomopathogenic nematodes vary between species, influencing their soil depth distributions and host preferences. Infective juveniles use strategies to find hosts that vary from ambush and cruise foraging. In order to ambush prey, some *Steinernema* species nictate, or raise their bodies off the soil surface so they are better poised to attach to passing insects, which are much larger in size. Many *Steinernema* are able to jump by forming a loop with their bodies that creates stored energy which, when released, propels them through the air. Other species adopt a cruising strategy and rarely nictate. Instead, they roam through the soil searching for potential hosts. These foraging strategies influence which hosts the nematodes infect. For example, ambush predators such as *Steinernema carpocapsae* infect more insects on the surface, while cruising predators like *Heterorhabditis bacteriophora* infect insects that live deep in the soil.

Table 1: Commercial use of entomopathogenic nematodes (EPN) *Steinernema* and *Heterorhabditis* as bio insecticides

EPN species	Major pest(s) targeted
<i>Steinernema glaseri</i>	White grubs (Scarabs, especially Japanese beetle, <i>Popillia</i> sp.), banana root borers
<i>Steinernema kraussei</i>	Black vine weevil, <i>Otiorynchus sulcatus</i>
<i>Steinernema carpocapsae</i>	Turfgrass pests- billbugs, cutworms, armyworms, sod webworms, chinch bugs, crane flies. Orchard, ornamental and vegetable pests - banana moths, codling moths, cranberry girdlers, dogwood borers and other clearwing borer species, black vine weevils, peach tree borers, shore flies (<i>Scatella</i> spp.)

*Steinernema feltiae*Fungus gnats (*Bradysia* spp.), shore flies, western flower thrips, leafminers**How to use Beneficial Nematodes**

The beneficial insect killing nematode *S. feltiae* is sold under the trade names of Nema Shield, Nemasys, Scanmask and Entonem. All of these products are labelled as a soil drench treatment against fungus gnat larvae. Preventative applications to moist soils work best.

- Apply nematodes with a sprayer (remove screens and filters), injector, hose end sprayer or even a watering can in very small operations.
- If using an injector, set the dilution to 1:100. Remove all filters or screens (50 mesh or finer) in any spray lines so that the nematodes can pass through unimpeded and undamaged.

Problems associated with nematode use**Timing**

Nematodes prey on the larval stage of most insects, so you must learn when larvae are present. On the lawn, grubs usually appear and feed on roots during the late summer and early fall. Apply nematodes at dawn or dusk, because high levels of ultraviolet rays from the sun can kill them.

Storage and Handling

Need to use live nematodes as soon as possible for maximum efficacy, because they have a limited shelf life. If stored properly in a refrigerator, they can live up to several months. Be careful with storage conditions, though, because extreme temperatures may kill nematodes or render them ineffective.

Environmental Conditions

Adequate moisture is the most important nematodes need water to move and find host insects. If they become dry, they could die. Nematodes move better in sandy soils with large pore areas than in clay soils. High salinity and high or low pH can also render these parasites ineffective. Some insecticides, such as those containing carbaryl, are toxic to nematodes. Before applying any lawn chemicals, check with your nursery about whether they are safe for nematodes.

CONCLUSION

In the present context of food and environmental safety, biological and non-chemical approaches for the management of insect pests are need of the hour. The high cost of chemical pesticides, its adverse effect on the environment and development of resistance against chemicals in the host demand an alternative approach for crop pests management, which should be eco-friendly and cost-effective. In this regard EPN's would be excellent bio control agents for the management of variety of insect pests and also one of the most efficient alternatives to chemical insecticides. The beneficial role of nematodes in agroecosystems has not received much attention as of plant parasitic group. But the presence of many groups of beneficial nematodes in to soil is vitally important in soil ecosystem process. The insect parasitic nematodes have been used for controlling the insect pests in industrialized countries but not in the developing countries. In the present context the two basic elements necessary for entomopathogenic nematodes to be successful are (i) a suitable nematode for the target pest, and (ii) favourable economics for its commercialization. For sustainable agriculture an integrated approach of all the methods are required to obtain maximum effect without interfering with the effectiveness of other practices. Since entomopathogenic nematodes can interact synergistically with several chemicals and bioagents a combination of multiple tactics should be prepared to achieve a satisfactory result. In the recent years some progress has been made in developing application technologies, however, further improvements are still needed to make entomopathogenic nematodes compete with other insecticides. Increase in shelf life of nematodes, improvement in transport logistic and marketing will substitute insecticides and contribute to stabilize agriculture environments and crop yields.

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