Entomopathogenic Nematodes as a Biological Weapon against Insect-pests

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Entomopathogenic nematodes (EPNs) are increasingly recognized as potent biological control agents against insect pests. These microscopic roundworms, naturally occurring in soil, infect and eliminate a broad variety of insect hosts. The utilization of EPNs as a biological weapon against insect pests offers a promising alternative to chemical pesticides, addressing concerns about environmental damage and pesticide resistance. EPNs have several advantageous traits. They demonstrate a preference for particular insect species, focusing on specific targets without harming non-target organisms, making them environmentally friendly. They are also capable of persisting in various environmental conditions, enhancing their efficacy as biocontrol agents.

Introduction

Nematodes belong to the most prevalent animal groups on the planet. Despite having extremely basic morphology, they have adapted to live in a variety of environments, including those that support invertebrates. Because they can spread diseases to humans and animals and have a negative economic impact on a variety of agricultural products, nematodes are typically regarded as pests. Nonetheless, there are a few but noteworthy instances of advantageous entomogenous nematodes, or nematodes connected to insects (typically in a parasitic manner). (Smith *et al*., 2020)

The word "entomopathogenic" comes from two Greek words, "*entomon*" means insect and "*pathogenic*" means "causing disease" (Gozel *al.,* 2016). The potential for these entomopathogenic nematodes (EPNs) to function as biological control agents of insect pests makes them highly interesting. A nematode can be an efficient biological control agent if it attacks an insect pest, kills or retards the growth of the insect host, and can reproduce in large quantities. Unlike their plant-eating distant relatives, entomopathogenic nematodes do not harm plants; they only attack potentially for insect-pests, particularly those that live in the soil.

More than 90% of insects, including many pest species, have at least one stage of their life cycle in the soil. Whenever the susceptible stage of insect-pests comes in realm of EPNs in soil, it permits the EPN to exploit a range of hosts that spans nearly all hosts. Especially, in cases, when this stage is larval or pupal, it inserts an opportunity for an effective management programme to be developed.

There are nine families of nematodes which include parasitic species that attack insects and kill or sterilize them, or alter their development (Ravichandra, 2013). In the context of Agriculture, only 2 families are of considerable importance *viz*., *Steinernematidae* and *Heterorhabditidae*.

Taxonomic position of EPNs

Kingdom: *Animalia*
Phylum: *Nematoda*
Class: *Chromadorea*
Order: *Rhabditida*

Family: 1. *Steinernematidae*: Genus: *Steinernema sp*.
2. *Heterorhabditidae*: Genus: *Heterorhabditidis sp*.

Life cycle

Under suitable environmental conditions, the infective juvenile (3rd IJ) nematodes seek insect larvae and pupae in the soil. They penetrate host insects either indirectly through natural openings of body (anus, mouth, and spiracles) or directly through the thin cuticular region and release a bacterium (*Xenorhabdus* for *Steinernema sp*., *Photorhabdus* for *Heterorhabditis sp*.) into the haemolymph, that kills the insects within a day or two. Insects killed by *Steinernema carpocapsae* are yellow to brown, and those killed by *Heterorhabditis bacteriophora* are reddish-brown. After the death of the host, the nematodes feed on the bacteria and insect body contents, and reproduce. Thereafter hundreds to thousands of infective juveniles (3rd infective juveniles) are released into the surrounding soil to search for new insect hosts and perpetuate their life cycle.

- *Infective juvenile stage*: The 3rd stage juvenile (IJ) is free-living and non-feeding in the soil, where it infects an insect host.
- *Host infection*: The IJ enters the host through natural openings like the mouth, anus, or spiracles. It then penetrates the midgut wall or tracheae into the insect's body cavity, releasing pathogenic bacteria into the haemolymph.
- *Development and reproduction*: The nematodes develop and reproduce in the insect, feeding on host tissues and bacterial cells. The affected host typically passes away within a day or two.
- *New infective juveniles*: As food resources are depleted, the nematodes complete 1–3 generations in the dead body of host and produce new infective juveniles.
- *Emigration*: Large numbers of infective juveniles emerge out from the insect cadaver to find new hosts.

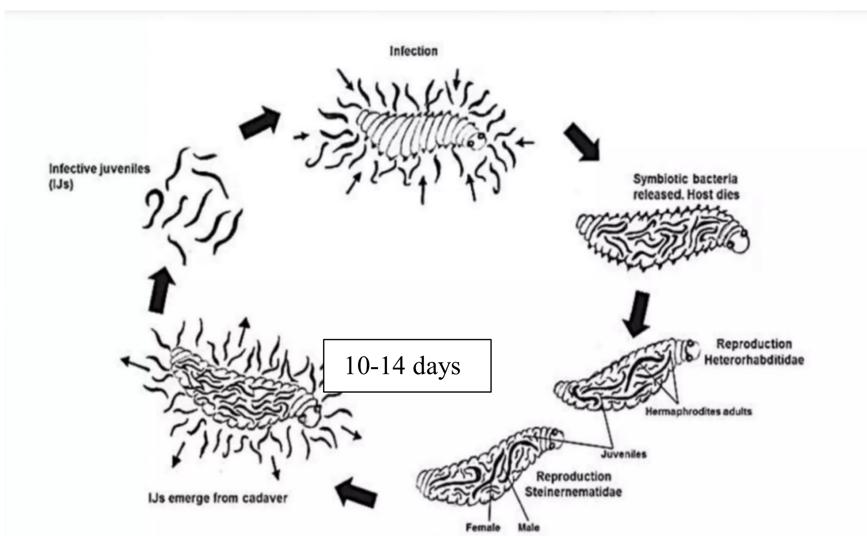

Fig. 1. General life cycle of Entomopathogenic Nematodes. (*Source*: Ramakuwela *et al*., 2015)

Nematode-bacteria symbiosis

The mutually beneficial relationship between entomopathogenic nematodes (EPNs) and their partner bacteria is a captivating instance of mutualism, where both partners benefit from their interaction. EPNs are dependent on specific bacteria from the genera *Xenorhabdus* (for *Steinernema* nematodes) or *Photorhabdus* (for *Heterorhabditis* nematodes) for their pathogenicity and reproduction. The relationship between parasitic nematodes and the respective bacteria is highly specific. Symbiotic bacteria of both genera are motile, gram negative and belong to the family Enterobacteriaceae (Ravichandra, 2013).

Entomopathogenic nematodes form a complex with bacteria, where the nematode may seem like just a biological syringe for the bacteria. However, their relationship exemplifies classic mutualism. The growth and reproduction of the nematode rely on the environment created in the host cadaver by the bacteria. (Poinar *et al*., 1990)

In addition, the bacterium produces anti-microbial that prevent rival secondary invaders from colonizing the cadaver and anti-immune proteins that help the nematode get past the host's defenses. Apart from that, the bacterium is not invasive and depends on the nematode to find and enter suitable hosts. Cadaver with *Heterorhabditis sp*. will be reddish-brown and those with *Steinernema sp*. will be yellow to brown due to the presence of pigment in respective bacteria. (Poinar *et al*., 2018)

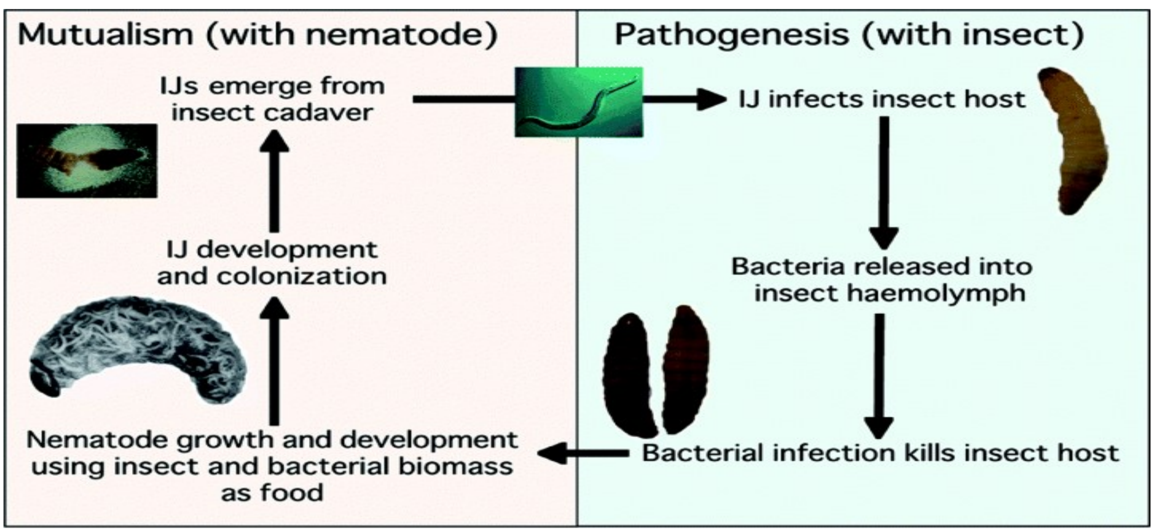

Fig. 2. Interaction of bacteria with EPN and host insect larvae. (*Source*: Kundu *et al.,* 2019)

Bacterial location in nematode: -

In *Heterorhabditis* sp., *Photorhabdus* bacteria reside in the last 2/3rd part of intestine.

In *Steinernema* sp. *Xenorhabdus* bacteria are present within specialised intestinal vesicle.

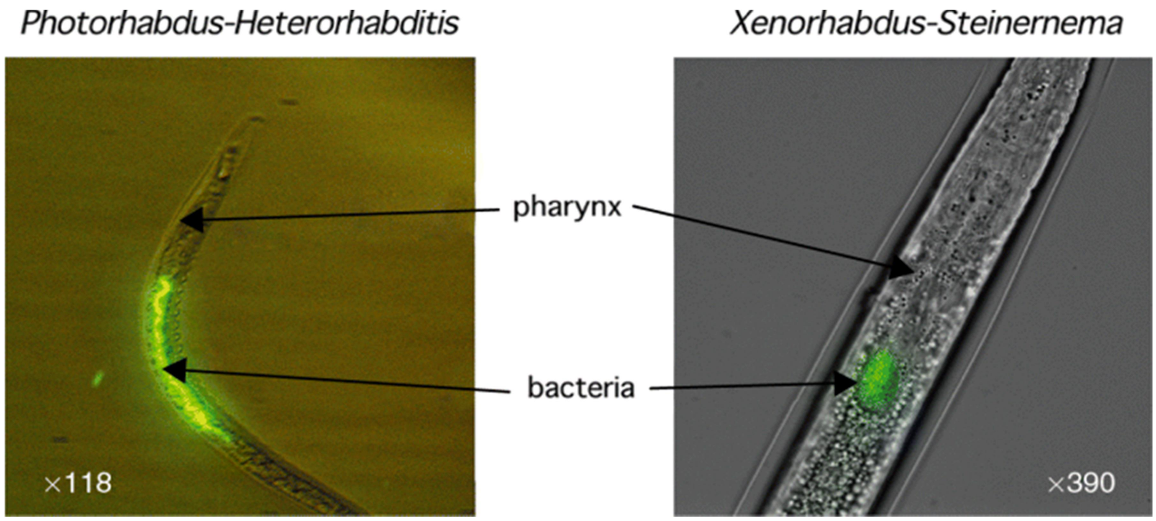

Fig. 3. Location of symbiotic bacteria in the respective nematode's gut. (*Source*: Goodrich-Blair *et al*., 2007)

Searching behaviour of EPNs

EPN species and strains differ in their activity against different insect pests. These differences are due to the different search behaviour of nematode species and also the type and number of bacteria carried within the infective juveniles. There are two major strategies used by EPNs to search for their host insect larvae or pupal stage:

- *Ambushers*: These EPN species uses an ambush (energy-conserving approach) approach to locate their insect hosts in the top layer of the soil. The infective juvenile nematodes stand on their tails and wait for long periods for an insect to come close enough to latch on and kill more mobile insects that live in the top layer of soil or thatch layer, including cutworms and armyworms e.g. *Steinernema carpocapsae.*
- *Cruisers*: These are usually underground and very active, travelling great distances to locate their host. Therefore, they are more efficient in targeting hosts that are less mobile, found deeper in the soil such as maggots, white grubs, and weevil larvae, e.g. *Steinernema glaseri, Heterorhabditis bacteriophora etc.*

Table 1. Some EPN species effective against major insect-pests in India

Crops	Insect-pests	EPNs
Rice, Sugarcane	*Scirpophagus incertulus*	*S. carpocapsae*
Maize	*Helicoverpa armigera*	*S. riobrave*
Cotton	*Spodoptera littoralis, Earias insulana*	*S. carpocapsae*
Tomato	*Agrotis ipsilon*	*S. bicornutum*
Brinjal	*Leucinoides orbonalis*	*S. carpocapsae, H. indica*
Pigeonpea	*H. armigera*	*H. indica*
Cabbage	*Plutella xylostella*	*H. bacteriophora*
Apple	*Cydia pomonella*	*S. carpocapsae*
Ornamental	Root weevil, Wood weevil	*H. bacteriphora, H. megidis*
Turf	Army worm, cut worm	*S. carpocapsae*
Sweet potato	*Cylas formicarius*	*S. carpocapsae*

Source: Abd -Elgawad *et al*., 2014

Table 2. Commercial EPN products available

Nematode species	Product formulation	Country
Steinernema carpocapsae	ORTHO bio-safe, Bio-vector, X GNAT	USA
S. feltiae	Magnet, Nemasys	UK
S. riobravis	Vector MG, Bio-vector	USA
S. megidis	Nemasys	UK
S. carpocapsae	Green commandos, Soil commandos	India
Heterorhabditis bacteriophora	Otinem	USA

Source: Alramadan *et al*., 2019

Application

Due to their ability to target a wide variety of pests, their simplicity in mass production and field application using standard equipment, EPNs are an excellent choice for pest management. EPN formulations applied directly to the soil, offer a more environment-friendly, chemical-free, possibility. Use a backpack sprayer to apply the EPN formulation, after mixing it with water (150 g EPN formulation/sprayer tank) (According to ICAR-SBI).

- *Apply at the right time*: Apply in the evening so the EPNs can activate at night without being damaged by sunlight.
- *Apply the right dose*: The recommended dose is 2 kg of powder formulation per acre or 1×10^8 Infective juveniles/acre.
- *Apply in the right areas*: Apply more concentrated EPNs in border rows, extending 5–7 meters inward.
- *Apply with the right equipment*: You can use backpack, hand, or tractor sprayers, sprinklers, or irrigation equipment to apply EPNs. Drip irrigation is the most common method of irrigation for applying EPNs, but you can also use furrow irrigation or mini-sprinkles.
- *Maintain soil moisture*: Ensure the treated area remains moist for at least five days after application. Nematodes need a thin layer of water to move, so maintaining the right moisture level helps them be more active and effective.
- *Consider the temperature*: Warmer temperatures reduce nematode survival, while cooler temperatures reduce their activity and infectivity. Therefore, a temperature range of 20-30 °C is ideal for the effectiveness of EPNs.

Advantages of EPNs

EPNs can be applied using standard agrochemical equipment, such as electrostatic fan, pressurized mist and aerial sprayers, without the need for specialized tools. They are highly effective against many significant soil insect pests, while remaining safe for plants and animals. Due to their safety profile, using nematodes is less hazardous than chemical treatments or even Bacillus thuringiensis applications. Additionally, they do not pose issues related to residues, re-entry time, groundwater contamination, and harm to pollinators or chemical trespass.

Most biological agents take days or even weeks to eliminate pests, while nematodes, in collaboration with their symbiotic bacteria, are able to kill insects within 24-48 hours. Numerous insect pests have the potential to spread infection, but field research has not revealed any negative consequences on non-targets. They are able to survive for longer period without host. Commercial mass production is possible either in live insects (*Galleria mellonella*) or fermenters. EPNs can be incorporated into Integrated Pest Management (IPM) because they are compatible with most chemical pesticides used in pest control. (Lacey *et al*., 2012)

Limitations of EPNs

Entomopathogenic nematodes are highly versatile and effective against various soil and hidden insect pests in different cropping systems, but they remain underused. Like other biological control agents, nematodes have limitations as they are living organisms that require specific conditions to function effectively. Therefore, insecticidal nematodes are quickly rendered inactive by desiccation or UV light; chemical insecticides face fewer restrictions. Nematodes also have a smaller temperature range of effectiveness than chemicals, and their effectiveness is more influenced by factors such as poor soil type, irrigation frequency and thatch depth (Hazir *et al.,* 2003).

Nematode-based insecticides can become inactive if stored in hot vehicles. They also can't be left in spray tanks for extended periods and are not compatible with several agricultural chemicals. Some species require different screen sizes, and some species cannot be applied with high-pressure application equipment. Nematodes that are not used the following year cannot be applied either. Although chemicals present issues like mammalian toxicity, resistance, and groundwater pollution, there is a substantial knowledge base supporting their use. To speed up the integration of nematodes into IPM systems, users need to become more informed about their effective application (Ravichandra, 2013).

Conclusion

EPNs offer a promising avenue for combating insect-pests in Agriculture and beyond. As evidenced by numerous studies, these microscopic organisms possess the ability to infect and eliminate a wide range of insect-pests, making them a potentially effective biological weapon in pest management strategies. The use of EPNs as a biological weapon against insect-pests holds significant potential for sustainable pest control, providing an eco-friendly alternative to chemical pesticides. Yet additional research is necessary to refine their application methods, enhance their efficacy against specific target pests and ensure minimal impact on non-target organisms and the environment.