Eco-Commandos Beneath the Surface: The Dual Strategy of Ambushing and Cruising Nematodes

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ABSTRACT

The need for eco-friendly approaches in agriculture is growing, such as using biopesticides, which have a low detrimental effect on the environment. Among them, entomopathogenic nematodes (EPNs) are effective tools for controlling insect pests. EPNs are microscopic, soil-dwelling roundworms naturally present in diverse agroecosystems and are distinguished by their capacity to infect and kill a broad spectrum of insect hosts. Their high host specificity, ecological compatibility and negligible effects on non-target organisms make them valuable components of Integrated Pest Management (IPM) systems. This article provides little information about the characteristics, mechanisms of action of EPNs. The review also integrates methodologies of isolating nematodes from soil and mass production on a small scale. It further examines their advantages and the challenges associated with their large-scale adoption and highlights their significance in promoting sustainable food production systems.

INTRODUCTION

Modern agriculture faces growing challenges arising from the intensive use of chemical pesticides, pest resistance, food safety concerns and environmental degradation. Although chemical pesticides are effective, their extensive use causes resistance, resurgence, leading to residue buildup and finally harming beneficial organisms that disrupt natural biological control systems. In

response to these issues, bio-input-based solutions have gained attention as a key strategy for promoting sustainable and environmentally responsible farming. Bio pesticides include living organisms or their metabolic products and microbial inoculants, which are effective in controlling the pests. Among these, entomopathogenic nematodes (EPNs) stand out as a scientifically proven group of bioinsecticides that offer both high efficacy and strong ecological compatibility.

Entomopathogenic nematodes (EPNs) are naturally occurring parasites found in soils worldwide, known for their remarkable ability to actively locate and infect insect hosts. They work in partnership with symbiotic bacteria species to rapidly kill their prey, often within 48 hours. EPNs are compatible with other IPM tools and have demonstrated synergistic effects when used in conjunction with other biocontrol agents, such as fungi and bacteria, resulting in increased pest mortality (Shapiro-Ilan and Dolinski, 2015). However, barriers to wider adoption remain, including challenges related to production costs, formulation stability and its dependency on weather parameters. Despite these obstacles, they are environmentally safe, can recycled in the soil and contribute to sustainable agriculture. Scientific research and decades of practical application have demonstrated that EPNs significantly suppress pest populations with minimal harm or no harm against non-target organisms. EPNs are valued for their broad host range, high virulence, safety for humans and animals and suitability for use with common agricultural equipment. Today, commercial EPN products are applied in orchards, vegetable fields, turfgrass and greenhouses.

Biological Classification of Entomopathogenic Nematodes

Kingdom: Animalia

Phylum: Nematoda

Class: Secernentea (also sometimes referred to as Chromadorea)

Order: Rhabditida

Suborder: Rhabditina

Infraorder: Panagrolaimomorpha

Entomopathogenic nematodes belong to two major families, Steinernematidae, represented by the genus *Steinernema* and symbiotically associated with *Xenorhabdus* spp. and Heterorhabditidae, represented by the genus *Heterorhabditis* and associated with *Photorhabdus* spp. These nematodes maintain a highly specific and obligate mutualistic relationship with their respective bacterial partners, with each nematode species typically harbouring a single, co-evolved bacterium within its gut or specialized intestinal vesicle.

Larvae infected with entomopathogenic nematodes exhibit distinct and characteristic color changes that help identify the specific nematode genus responsible for infection. Infections caused by *Steinernema* species typically result in larvae turning **creamy beige to dark brown,** a coloration produced by the growth of *Xenorhabdus* bacteria, which secrete pigmented metabolites as they multiply within the hemocoel. So*, Steinernema* species are commonly called black commandos, black knights or brown commandos because larvae infected by them typically turn brown to dark brown. In contrast, larvae infected by *Heterorhabditis* species develop a **brick-red to deep reddish-brown** color due to the proliferation of *Photorhabdus* bacteria, which release red pigments and cause rapid decomposition of host tissues. So, *Heterorhabditis* species are popularly known as red warriors, red killers or red commandos as infected larvae develop a striking brick-red or deep red. These visual changes arise from bacterial toxins, enzymatic degradation of

internal organs and the accumulation of metabolic by-products, all of which reflect the coordinated pathogenic activity of the nematode bacterium complex. The resulting pigmentation serves as a reliable diagnostic indicator for distinguishing between *Steinernema* and *Heterorhabditis* infections.

Lifecycle of EPNs

Entomopathogenic nematodes (EPNs), primarily belonging to the genera *Steinernema* and *Heterorhabditis*, are microscopic roundworms that act as obligate parasites of insects, forming a mutualistic association with symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively. Their life cycle consists of several distinct stages: egg, four juvenile stages (J1–J4) and adult (Fig. 1). Among these, the third-stage juvenile, known as the infective juvenile (IJ or J3), represents a specialized, non-feeding and developmentally arrested Dauer-like form responsible for locating, infecting and transmitting the symbiotic bacteria to suitable insect hosts. The IJs actively search for hosts in the soil using strategies such as ambushing or cruising, entering the insect body through natural openings (mouth, spiracles or anus) or thin areas of the cuticle. Once inside the hemocoel, the IJs release their symbiotic bacteria, which multiply rapidly, produce toxins and suppress the host's immune system, causing septicemia and death within 24 to 48 hours. The nematodes then feed on the bacterial biomass and decomposed host tissues, completing their remaining juvenile molts and maturing into adults, which reproduce within the insect's cadaver. Depending on the resources available, one or multiple generations may develop within the host before nutrient depletion triggers a new generation of IJs. These IJs reassociate with their bacterial symbionts, exit the cadaver and re-enter the soil to seek new hosts, thereby perpetuating the highly coordinated and efficient EPN–bacteria lifecycle (Lortkipanidze *et al.,* 2016).

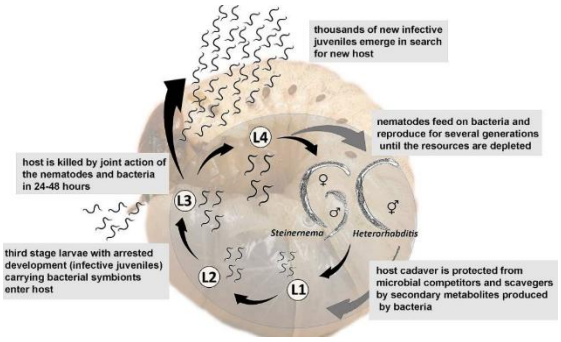

Fig. 1. Generalized life cycle of entomopathogenic nematodes

Searching strategies

Entomopathogenic nematodes (EPNs) employ two primary host-seeking strategies, called as Ambushers and Cruisers, each representing distinct behavioural adaptations shaped by soil ecology and host availability.

Ambushers exhibit limited horizontal movement, instead positioning themselves near the soil surface in a nictitating or standing posture, where they depend on direct contact with actively moving insect hosts. Many ambushers can perform rapid, spring-like jumps, allowing them to physically attach to passing insects and making this strategy particularly effective against surface-dwelling or transient larvae.

In contrast, **Cruiser** engages in continuous, directional movement through soil pores, actively searching for less mobile or concealed hosts. Their foraging behaviour is strongly influenced by chemotactic and mechanosensory cues, including carbon dioxide gradients, host volatiles and frass deposits that indicate host presence. These two contrasting strategies reflect the ecological flexibility of EPNs, enabling them to effectively exploit a wide range of host microhabitats by optimizing encounter rates through unique combinations of behavioural

and physiological traits (Lortkipanidze *et al.,* 2016).

In-Vivo Production of Entomopathogenic Nematodes Using *Galleria mellonella* Larvae

In-vivo production of entomopathogenic nematodes (EPNs) is commonly performed using the larvae of the Greater wax moth, *Galleria mellonella*. These larvae are highly susceptible to EPN infections, making them an ideal host for nematode reproduction. *Galleria mellonella* can be reared on a semi-synthetic diet, which simplifies their cultivation and reduces production costs. The host's body provides a nutrient-rich environment for the nematodes and their symbiotic bacteria, resulting in a high yield of infective juveniles (Kotchofa,and Baimey, 2019).

Semi-Synthetic Diet Recipe for Rearing *Galleria mellonella* Larvae

Ingredients for 1000 larvae:

- **Wheat flour**: 200g
- **Maize flour**: 200g
- **Milk powder**: 200g
- **Yeast powder**: 20g
- **Honey**: 200ml
- **Glycerine**: 150ml
- **Streptomycin**: 0.5g
- **Vitamin E**: 2 capsules

This semi-synthetic diet provides a balanced nutritional profile to support the growth and development of *Galleria mellonella* larvae, ensuring high yields for in-vivo production of entomopathogenic nematodes (Kotchofa,and Baimey, 2019).

Methods of Isolating Entomopathogenic Nematodes (EPNs) from Soil

In-situ Trap Baiting

In-situ trap baiting is a simple and effective field-based technique used to detect EPNs directly within their natural habitat. A 100-mL plastic container perforated with multiple small holes and properly labeled is prepared and lined from all sides with Whatman filter paper. A healthy 4th-instar *Galleria mellonella* larva is placed inside and the container is suspended using a 30 cm thread or wire so that it can be positioned within the rhizosphere or other moist soil regions where nematodes are likely to occur. Moisture in the soil stimulates nematode activity and if EPNs are present, they penetrate and infect the bait larva. After about one week, the bait containers are retrieved and larvae showing signs of infection are taken to the laboratory for harvesting of EPNs.

Soil Collection Method

In this method, soil samples are collected from multiple locations, especially from moist zones near plant root regions where EPN populations are most active. Approximately 1000 g of soil is collected at each sampling point and stored in clean polythene bags. In the laboratory, a tray setup is prepared by alternating layers of two to three cm soil with three to four *Galleria mellonella* larvae, repeating the sequence three times and finishing with a top layer of soil. The setup provides an ideal environment for nematodes, if present in the sample, to seek out and infect the larvae. After several days, the larvae are checked for infection symptoms, changes in color, texture or rigidity and infected larvae are separated for nematode extraction.

Collection of EPNs from Dead Cadavers
Once infected larvae are identified; they serve as a biological source for harvesting

nematodes. The Modified White Trap method is commonly used for this purpose.

Modified White Trap Method

The modified White trap technique provides a controlled environment that encourages infective juveniles (IJs) to emerge from the insect cadaver. To set up the trap, a sheet of white filter paper or paper towel is placed across the bottom of a Petri dish or shallow tray. A small elevated platform, such as an inverted lid, is positioned in the centre, allowing the filter paper to form a gentle bridge from the platform to the edges of the dish. Infected *Galleria* larvae are placed on this platform using sterilized forceps, ensuring the larvae are not submerged when water is added to the dish. Water is poured until it reaches just below the filter paper, keeping it uniformly moist. This moist microenvironment stimulates the newly formed IJs to emerge from the cadaver, move through the damp filter paper and accumulate in the surrounding water. The dish is kept covered in a cool, dark place for 5–7 days to promote optimal emergence. The water containing the IJs is then collected gently using a pipette and can be replenished periodically to maximize yield. Harvested nematodes are stored in distilled water or Ringer's solution under cool conditions to maintain viability.

Inoculation of EPNs for Small-Scale Mass Multiplication

For small-scale mass production, *Galleria mellonella* larvae serve as an efficient and economical host. A sterile Petri plate is lined with moistened filter paper and approximately six healthy larvae are placed evenly on its surface. A 1-mL suspension of active infective juveniles is then pipetted onto the larvae and the surrounding filter paper, ensuring uniform distribution. As infection progresses, the larvae gradually weaken and cease feeding within 24–48 hours. Over the next 5 to 7 days, the characteristic color change often reddish or brownish, depending on the EPN species indicates successful infection and nematode development within the insect body. These cadavers are subsequently transferred to a modified White trap setup for harvesting newly formed IJs. This method allows continuous and efficient multiplication of EPNs on a small scale for laboratory studies or field applications.

Formulation and storage

Formulation and storage of entomopathogenic nematodes (EPNs) are critical processes that maintain the viability, infectivity and stability of infective juveniles (IJs) from production to field application. Because IJs are highly sensitive to desiccation, heat and ultraviolet light, formulations are designed to create a protective microenvironment that preserves moisture, aeration and physiological quiescence. EPNs are commonly incorporated into aqueous suspensions, alginate or polymer-based gels, inert carriers such as sponge, clay, peat, or vermiculite, as well as water-dispersible or oil-based granules. During preparation, IJs are gently mixed with these inert materials under controlled humidity and temperature to avoid mechanical stress; for example, in granular formulations, the nematodes are blended with moistened carriers using low-speed mixers to ensure uniform distribution, while gel or alginate formulations involve encapsulating the nematodes within cross-linked polymer matrices that maintain hydration and slow release. Storage typically requires maintaining low temperatures (4–10 °C), stable moisture levels and sufficient oxygen availability to suppress metabolic activity without inducing stress-related mortality. Additives such as cryoprotectants, osmotic stabilizers and antimicrobial agents may be included to enhance shelf life and preserve the integrity of the symbiotic bacteria. Altogether, successful formulation and storage depend on optimizing

environmental conditions and carrier compatibility to ensure long-term survival and effective delivery of EPNs for biological control (Cruz- Martinez *et al.,* 2017).

Pest Management Applications

Entomopathogenic nematodes (EPNs) have gained global recognition as effective biological control agents and their success is well documented through both laboratory studies and field applications. These nematodes, primarily from the genera *Steinernema* and *Heterorhabditis*, parasitize a wide range of soil-dwelling and concealed insect pests by releasing symbiotic bacteria that rapidly kill the host. Internationally, *Steinernema carpocapsae* has been used effectively against cutworms (*Agrotis spp.*) and armyworms, with laboratory assays consistently reporting rapid larval mortality within 24 to 48 hours. *Heterorhabditis bacteriophora* has demonstrated strong efficacy against white grubs (*Popillia japonica* and *Holotrichia spp.*), supported by both field trials and controlled experiments showing significant declines in grub populations. In greenhouse and mushroom house systems, *Steinernema feltiae* is widely used to manage fungus gnats (*Bradysia spp.*), with early instar larvae exhibiting high susceptibility in laboratory studies. In India, EPN-based pest management has also been extensively explored. *Heterorhabditis indica*, a species native to Indian soils, has shown excellent control of sugarcane early shoot borer (*Chilo infuscatellus*) and root grubs such as *Holotrichia serrata*, with multiple ICAR-supported studies reporting high larval mortality and improved crop stand. *Steinernema thermophilum*, another Indian isolate adapted to higher temperatures, has been effective against the sorghum shoot fly, cabbage root fly larvae and various coleopteran pests in vegetable systems. Additionally, Indian laboratory trials have demonstrated strong pathogenicity of *S. abbasi* and *H. indica* against pests like the red palm weevil (*Rhynchophorus ferrugineus*) and sweet potato weevil (*Cylas formicarius*) (Raypuriya *et al.,* 2025)

Challenges and Limitations

Despite their proven efficacy and environmental safety, the large-scale adoption of entomopathogenic nematodes (EPNs) in pest management faces several practical, biological and economic challenges that limit their wider use. One major limitation is their sensitivity to environmental factors, particularly temperature extremes, desiccation and ultraviolet radiation, which restrict their performance under open-field conditions unless adequate soil moisture and moderate temperatures are maintained. Farmers may also show limited adoption due to higher initial costs, lack of awareness and the perception that biocontrol agents act more slowly than chemical pesticides. Finally, large-scale production requires sophisticated in vivo or in vitro culturing techniques and maintaining the stability of the nematode–bacterium symbiosis during mass production poses additional technical challenges. Collectively, these constraints highlight the need for improved formulations, cost-effective production systems, enhanced field-delivery technologies and broader farmer education to realize the full potential of EPNs in sustainable pest management (Campos-Herrera *et al.,* 2025).

CONCLUSION

Entomopathogenic nematodes represent a powerful, ecologically compatible tool for sustainable insect pest management, offering a promising alternative to conventional chemical pesticides. Their unique ability to actively seek, infect and rapidly kill a wide range of insect hosts—while remaining safe for humans, animals and beneficial organisms—makes them highly suitable for integration into

modern IPM programs. Although challenges such as environmental sensitivity, formulation limitations and production costs continue to constrain large-scale adoption, ongoing advancements in biotechnology, mass-rearing techniques and improved delivery systems are steadily enhancing their reliability and field performance. With continued research, farmer awareness and refinement of application strategies, EPNs have the potential to play an increasingly significant role in reducing chemical pesticide dependence and supporting resilient, sustainable agricultural production systems.

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