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Tiny warriors: Harnessing the power of entomopathogenic nematodes in biological pest control

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Abstract
The Entomopathogenic nematodes (EPNs) are soil inhabiting, delicate or soft bodied, non-segmented roundworms that are obligate or facultative parasites of insect pests. Heterorhabditidae and Steinernematidae are two EPN families that are widely spread in a variety of soil types. EPNs kill insect hosts due to mutualistic bacteria present in nematode gut. Infective juveniles (IJs) pierce the host and then enter the hemocoel, releasing symbiotic bacteria that proliferate and ultimately kill the host due to septicemia. Their ability to target a broad spectrum of insect pests with specificity, combined with their safety for non-target organisms and the environment, makes them an attractive alternative to chemical pesticides. The diverse application methods and formulations available for EPNs enhance their adaptability to various agricultural and horticultural ecosystem, allowing for their integration into sustainable pest management strategies. However, the effectiveness of EPNs is influenced by several factors, including environmental conditions, formulation, and application techniques.

Keywords: Entomopathogenic nematodes (EPNs), Sustainable pest management and Infective juveniles (IJs)

Introduction

Annually insect pests cause serious damage to agricultural crops, which leads to substantial financial losses in agriculture worldwide. The farmers to combat these problems have become more reliant on chemical pesticides. Although chemical pesticides have played a significant role in managing important pests of crops, indiscriminate use of these pesticides has led to environmental pollution, development of insecticide resistance, secondary pest outbreaks, pesticide residues, and adverse effects on human health and other non-target organisms [1]. Natural approaches to managing target pests in agriculture/horticulture gained interest because of these negative impacts and the current status of chemical pesticides [2-3]. Natural approaches are based on the development and integration of more eco-friendly alternatives in pest management practices among which Biological control is one such alternative [4-5]. Biological control is defined as "the management of a pest by the deliberate use of living organisms (natural or applied) to maintain the pest population density at a lower level than would occur in the absence of the biological agent" [6].

The microorganisms such as fungi, bacteria, viruses, entomopathogenic nematodes (EPNs) and protozoa have shown their potential use in biological control of insect pests [7-8]. Among these, EPNs are effective biocontrol agents for the control of insect pests, especially soil inhabiting insects. EPNs are microscopic, parasitic roundworms that have evolved as natural enemies of a wide variety of insect pests. Forty nematode families have been found associated with insects, only two families: Heterorhabditidae and Steinernematidae are more effective and widely been used as biological control agents for insect pests [9]**.** Due to special qualities like wide host range, wide distribution and quick action. The symbiotic relationship with entomopathogenic bacteria, Photorhabdus and Xenorhabdus genera for Heterorhabditis and Steinernema, respectively boosts their efficacy. However, their effectiveness is mostly dependent on the presence of particular conditions like as high relative humidity, low temperature and sunlight (UV radiation). Nematodes of the genera Heterorhabditis and Steinernema are found worldwide, infecting about 250 distinct insect species belonging to ten orders [10]. Out of the 113 species that are known worldwide, only 17 recognized species (3 Heterorhabditis and 14 Steinernema) have been described in India.

The efficiency of EPNs against a wide range of insect pests, including those that are soil-dwelling or have a stage of their life cycle in the soil, makes them highly desirable for use in integrated pest management (IPM) programs [11]. Their capacity to eliminate pests while having the least negative effects on humans and beneficial insects makes them especially valuable. EPNs are also useful for large-scale pest management in agricultural environments since they can be mass-produced. Their use is expanding globally as an eco-friendly alternative to chemical pesticides, which have raised concerns due to their environmental persistence, development of pest resistance, and harmful effects on non-target species. The versatility and efficacy of EPNs, coupled with their minimal environmental footprint, make them a promising tool in sustainable agriculture [12].

Biology and life cycle

The EPNs have simple life cycle, *viz.*, egg, four juvenile stages (separated by moults) and adult stages. The infective stage is J3 (third juvenile stage), and it is known as infective juveniles (IJs) [13]. The IJs are non-feeding, free-living active stage capable of withstanding adverse environmental circumstances and the non-availability of host for an extended time period. The IJs enters the host through the natural openings like the mouth, spiracles, anus, or by direct cuticle penetration (Heterorhabditis). After penetrating the host body, the nematode discharges bacteria from its lumen, which grow rapidly in the host hemolymph, causing septicemia [14] and resulting in the host's death. Later, the nematode and bacterium feed on the host tissues, reproduce and multiplies until the host tissues deplete and emerge as IJs, which seek a new host. Under ideal circumstances, one life cycle for both Heterorhabditids and Steinernematids inside a host takes three to seven days. Nematodes of the families Heterorhabditidae and Steinernematidae reproduce in distinct ways. IJs of *Heterorhabditis* nematodes grow into hermaphroditic adults, but the following generation produces both males and females, whereas IJs of *Steinernema* generate both males and females in all generations (gonochorism) [15]. The cadaver turns red when Heterorhabditids kill the insect hosts, but the body turns tans or brown when Steinernematids kill the insect hosts [16].

Host-Seeking Strategies and Cues

EPNs use a variety of strategies to locate their insect hosts, ranging from ambushing (waiting for the host) to cruising (actively seeking hosts). EPNs that employ the ambushing approach typically stay motionless at or close to the soil's surface and find their hosts by making direct contact with them. Insect pests that are extremely mobile near the soil surface, such cutworms, armyworms, and mole crickets, are best controlled by ambusher EPNs. Cruising is EPNs that use the cruising strategy are highly mobile and able to move throughout the soil profile. Cruisers locate their host by chemical cues. Chemical cues are grouped into three main types: CO_2, plant-derived, and insect-derived chemicals [17]. These cues are integrated hierarchically by EPNs, depending on the species, age, experience, and environmental conditions.

1. **Carbon Dioxide (CO_2) Cues:** CO_2 is a ubiquitous cue used by EPNs to detect the presence of potential hosts. The response to CO_2 varies among different EPN species and foraging strategies. CO_2 detection is mediated by specific sensory neurons and can be used in combination with other chemical cues [17-18].

2. **Plant-Derived Cues:** EPNs can detect herbivore-induced plant volatiles (HIPVs), which are released by plants in response to herbivore attack. These volatiles help EPNs locate areas where herbivores are likely to be present [19].

3. **Insect-Derived Cues:** Insect excretions, pheromones, and other waste products serve as cues for EPNs to locate hosts. The specific compounds released by insects can either attract or repel EPNs, and these interactions are crucial for successful host infection [17].

Host range

Nematodes species	Symbiotic bacteria	Major targeted insects	Reference
S. abbasi	*X. indica*	Lepidopteran pests	[20]
S. glaseri	*X. poinarii*	White grubs including Japanese beetle, scarabs, Popillia spp. and banana root borers	[21]
S. thermophilum	*X. bacteria*	Mealybug, Aphid and whitefly	[22]
S. feltiae	*X. bovienii*	Bradysia spp. (Fungus gnats), western flower thrips, leafminers, shore flies	[23]
S. minutum	*Xenorhabdus spp.*	*H. armigera, Ostrinia furnacalis* and *S. litura*	[24]
S. carpocapsae	*X. nematophila*	Pests of vegetables and ornamentals (codling moths, banana moths, dogwood borer, cranberry girdlers, peachtree borers, black vine wee- vils, shore flies) and pests of urfgrass (armyworms, cutworms, sod webworms, chinch bugs, billbugs, crane flies)	[23]
S. riobrave	*X. cabanillasii*	Mole crickets and citrus root weevils (*Diaprepes* spp.)	[25]
S. kraussei	*Xenorhabdus spp.*	Otiorhynchus sulcatus (Black vine weevil)	[26]
H. indica	*P. luminescens akhurstii*	Root mealy bugs, fungus gnats, and grubs	[21]
H. marelatus	*P. tasmanensis*	Scarabs, black vine weevils, cutworms	[27]
H. bacteriophora	*P. luminescens luminescens*	Black vine weevils, scarabs, and cutworms	[28]
H. megidis	*P. temperate*	Weevils	[23]

EPN Strategies for Immunosuppression of host

Entomopathogenic nematodes (EPNs) employ several strategies to suppress the immune system of their insect hosts [29], ensuring successful infection and subsequent death. These strategies include:

1. **Symbiotic Bacteria Release:** EPNs harbor symbiotic bacteria (e.g., *Xenorhabdus* and *Photorhabdus*) which are released into the insect's hemocoel. These bacteria produce toxins that suppress the host's immune response [29].
2. **Avoidance of Immune Recognition:** EPNs and their symbiotic bacteria can evade detection by the host's immune system, reducing the likelihood of an immune response [30].
3. **Secretion of Immunosuppressive Molecules**: EPNs secrete proteins and other molecules that directly inhibit the

host's immune pathways, including the suppression of antimicrobial peptides and other defence mechanisms [31].

4. **Inhibition of Hemocyte Function**: Hemocytes, which are crucial for cellular immunity in insects, are targeted and their functions are inhibited, preventing effective encapsulation and phagocytosis of the nematodes [32].

Culturing of EPNs

Culturing of EPNs involves several techniques, each tailored to specific requirements for research or large-scale production. Here are some commonly used methods:

1. ***In vivo* Culturing:** This traditional method involves infecting live insect hosts (like *Galleria mellonella* larvae) with EPNs. After infection, the nematodes reproduce inside the host, and infective juveniles emerge from the cadaver, which can be harvested. This method is simple and widely used for small-scale production and research [33].

2. ***In vitro* Solid Culture:** EPNs can be cultured on solid media that mimic the insect host environment. Agar-based media are supplemented with nutrients like ground liver, soy flour, or yeast extract. This method allows for higher yield than *in vivo* methods but requires careful management of environmental conditions (e.g., moisture and temperature) [34].

3. ***In vitro* Liquid Culture:** In this method, EPNs are cultured in a liquid medium that contains nutrients to support their growth. Liquid culture is suitable for large-scale industrial production and allows for the continuous harvesting of nematodes. The process involves a bioreactor where the culture conditions can be tightly controlled to optimize nematode yield and health [35].

4. **Two-Phase Culture:** This method starts with a solid phase (e.g., agar plates with nutrients) where nematodes are initially cultured. After initial growth, the nematodes are transferred to a liquid phase for further development. This hybrid method combines the benefits of both solid and liquid culturing techniques and can result in high nematode yields [36].

5. **Monoxenic Culture**: EPNs are cultured with their symbiotic bacteria in a controlled environment. The bacteria are essential for nematode growth and reproduction. Monoxenic cultures are used for both research purposes and production, ensuring that nematodes retain their virulence and effectiveness [37].

6. **Mass Rearing on Artificial Diets:** Researchers have developed artificial diets that mimic the host insect's tissue, allowing for large-scale EPN production without the need for live hosts. These diets often contain animal by-products, plant materials, and microbial additives. This method is particularly useful in commercial settings where high volumes of nematodes are required [38].

Formulations of EPNs

Entomopathogenic nematodes (EPNs) can be formulated in various ways to ensure their viability, ease of application, and effectiveness in pest control. Different formulations are designed to protect the nematodes during storage, transport, and application, and to optimize their release and activity in the target environment. Here are some common formulations of EPNs:

1. **Aqueous Suspension:** EPNs are suspended in water or a buffered solution and are typically used for immediate application. This formulation is straightforward and commonly used for soil drenching, foliar spraying, and application through irrigation systems. Aqueous suspensions are easy to apply but have a limited shelf life and require proper storage conditions to maintain nematode viability [39].

2. **Clay-Based Formulations:** EPNs are mixed with clay or other inert powders that can be stored dry and rehydrated before application. The clay helps protect the nematodes from desiccation and environmental stresses, allowing for extended shelf life and easy transport. Clay-based formulations are often used for soil applications and can be mixed with water for spraying [39].

3. **Gel Formulations:** EPNs are embedded in a gel matrix that retains moisture and protects them from environmental conditions like UV light and desiccation. Gel formulations are particularly useful for foliar applications and localized treatments, where the gel helps keep the nematodes on the plant surface for longer periods. This method also allows for slow release of nematodes over time [40].

4. **Alginate Capsules:** EPNs are encapsulated in alginate beads, which provide a protective barrier against environmental stresses and help in the controlled release of nematodes. Alginate capsules are typically used in situations where precise delivery of nematodes is required, such as in seed treatments or spot applications [41].

5. **Wettable Powders:** EPNs are formulated as pastes or wettable powders that can be easily mixed with water prior to application. These formulations allow for concentrated storage and can be used in various application methods, including spraying and drenching. They are designed to be user-friendly and to maintain nematode viability during storage [39].

6. **Cadaver-based formulations:** It involve using insect cadavers infected with EPNs as a means of applying these biological control agents to target pest populations. This method capitalizes on the natural lifecycle of EPNs, which reproduce inside an infected host and emerge as infective juveniles. Cadaver-based formulations are a cost-effective and environmentally friendly option for pest control in various agricultural settings [42].

Advantages of Using Entomopathogenic Nematodes (EPNs):

- **Eco-Friendly:** EPNs are natural biocontrol agents that reduce the need for chemical pesticides, minimizing environmental contamination and harm to non-target organisms.
- **Target-Specific:** They primarily target specific insect pests, reducing the risk of affecting beneficial insects, pollinators, and other non-target species.
- **Safety:** EPNs are safe for humans, animals, and plants, making them suitable for use in organic farming and residential areas.
- **Broad Spectrum of Action:** Effective against a wide range of soil-dwelling and foliar insect pests, including grubs, weevils, caterpillars, and more.

- **No Resistance Development:** Insects are less likely to develop resistance to EPNs compared to chemical pesticides, maintaining their effectiveness over time.
- **Compatibility with IPM:** EPNs integrate well with other integrated pest management (IPM) strategies, offering a holistic approach to pest control.

Problems Associated with the Use of Entomopathogenic Nematodes

- **Environmental Sensitivity:** EPNs are sensitive to environmental conditions such as temperature, moisture, and UV exposure, which can limit their effectiveness if conditions are not optimal.
- **Storage and Shelf Life:** Maintaining the viability of EPNs during storage and transport can be challenging, as they require specific conditions to remain effective.
- **Cost of Production:** While effective, EPNs can be more expensive to produce and apply than some chemical alternatives, especially at large scales.
- **Limited Efficacy in Dry Conditions:** EPNs require adequate moisture to move and infect hosts, making them less effective in arid or drought-prone environments.
- **Variability in Performance:** The effectiveness of EPNs can vary depending on the pest species, environmental conditions, and the specific nematode species used.

Conclusion

Entomopathogenic nematodes (EPNs) represent a powerful and versatile tool in the realm of biological pest control. Their ability to target a broad spectrum of insect pests with specificity, combined with their safety for non-target organisms and the environment, makes them an attractive alternative to chemical pesticides. The diverse application methods and formulations available for EPNs enhance their adaptability to various agricultural and horticultural settings, allowing for their integration into sustainable pest management strategies. However, the effectiveness of EPNs is influenced by several factors, including environmental conditions, formulation, and application techniques. Challenges such as sensitivity to environmental extremes, storage constraints, and higher costs compared to chemical pesticides must be addressed to maximize their potential. Future research and technological advancements are likely to improve the viability and accessibility of EPNs, paving the way for their broader adoption in integrated pest management programs.

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