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Original Research Paper

Developing Biopesticides Using Entomopathogenic Nematodes: Current Trends

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Abstract: Introduction: EPNs have emerged as vital biological control agents, with their ability to infect and kill insect hosts through mutualistic bacterial partners. This natural mode of action is particularly beneficial in sustainable agriculture, offering an eco-friendly solution to pest outbreaks. Results: Advances in biotechnology have enhanced the virulence and stability of EPNs, enabling more effective field applications. Novel delivery systems, such as encapsulation and gel formulations, have improved the survival and persistence of EPNs in various environmental conditions. The commercial production of EPNs has scaled up, making these biopesticides more accessible to farmers. Case studies from different regions demonstrate their successful integration into pest management programs, with reduced pest populations and minimal impact on non-target organisms. Conclusion: EPN-based biopesticides offer a powerful tool for sustainable pest control, with significant potential for expansion into various agro-ecosystems. Despite challenges such as environmental variability and regulatory constraints, the future of EPNs in biological control is promising. Continued research into strain improvement, formulation technologies, and synergistic approaches will further enhance their role in global pest management strategies.

Keywords: Biopesticides, Nematodes, Entomopathogenic, Agriculture, Pest Control, Formulation Resistance, Environment Technology, Vector Organic.

I. Introduction

Entomopathogenic nematodes (EPNs) have garnered significant attention as biological control agents, offering a promising alternative to chemical pesticides in the management of insect pests. EPNs, particularly those from the genera *Steinernema* and *Heterorhabditis*, are natural parasites of insects and have been explored for their potential in pest control due to their symbiotic relationship with entomopathogenic bacteria, primarily *Xenorhabdus* and *Photorhabdus*. These bacteria are essential for the pathogenicity of EPNs, as they help kill the host insect after nematode invasion [1]. The insect host, once infected by EPNs, typically succumbs within 24 to 48 hours due to septicemia caused by the bacterial symbionts. This process makes EPNs highly effective agents in reducing pest populations in a variety of agroecosystems. EPNs have been under investigation for pest control since the 1970s, and over the decades, considerable advancements have been made in both their understanding and practical application. The interest in using EPNs as biopesticides has risen due to increasing concerns over the environmental and health impacts of chemical pesticides [2]. Traditional chemical pest control methods have often led to negative consequences such as pesticide resistance, pollution, and detrimental effects on non-target organisms.

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EPN-based biopesticides offer a sustainable and eco-friendly alternative. Their ability to target a wide range of insect pests, combined with their safety for humans, animals, and plants, makes them ideal candidates for inclusion in integrated pest management (IPM) strategies. One of the key factors that makes EPNs so attractive for pest control is their highly specific mode of action [3]. EPNs locate their insect hosts through a combination of chemical cues and physical contact, entering the host through natural openings like the mouth, spiracles, or anus. Once inside, the nematodes release their symbiotic bacteria, which rapidly multiply and produce toxins that kill the host. The bacteria also break down the host tissues, providing nutrients for the nematodes to reproduce. After consuming the host, the next generation of nematodes exits the dead insect to seek new hosts [4]. This natural cycle allows EPNs to self-replicate and sustain their population in the environment, making them a cost-effective solution in long-term pest control. Despite the promising potential of EPNs, several challenges have been encountered in their development and widespread use as biopesticides. One of the primary obstacles is the difficulty of mass-producing and formulating EPNs in a way that preserves their viability and efficacy during storage and transport. EPNs are living organisms, and maintaining their survival outside of a host environment can be challenging [5].

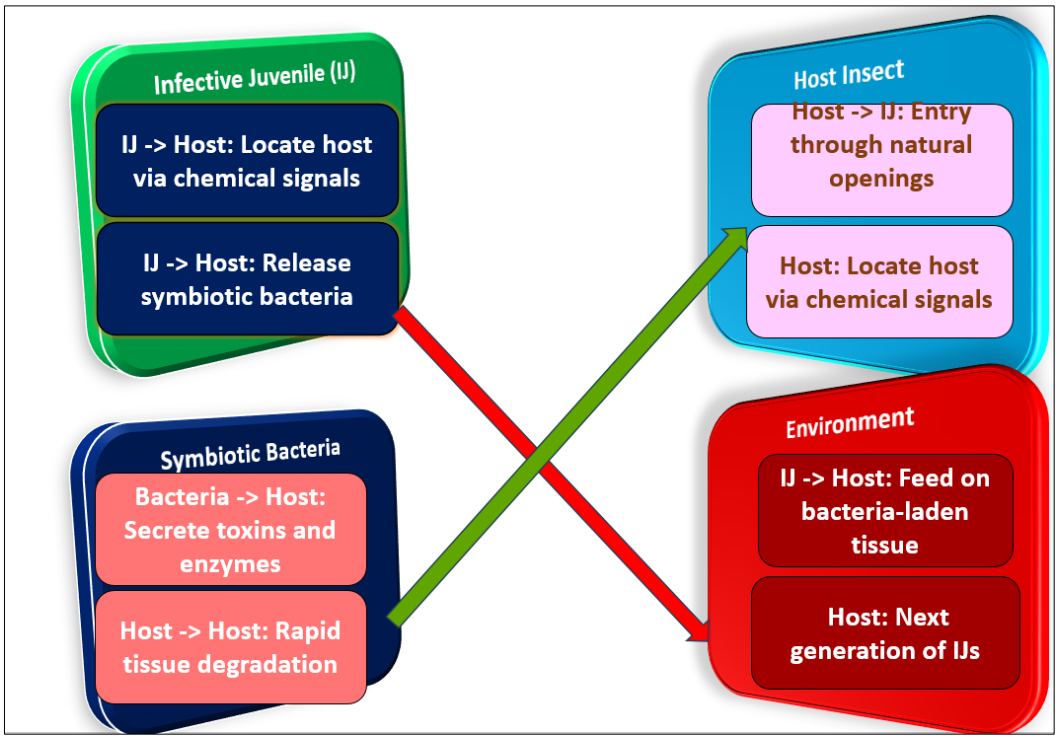


Figure 1. Mechanisms of EPNs in Pest Suppression

Advances in biotechnological research have addressed some of these issues, leading to the development of novel formulations that enhance the stability and shelf-life of EPNs. Techniques such as encapsulation in gels, or the use of carrier materials like clay or vermiculite, have been explored to protect the nematodes during storage and application. Another challenge is ensuring the effectiveness of EPNs in diverse field conditions. Environmental factors such as soil moisture, temperature, and UV exposure can significantly impact the survival and performance of EPNs when applied in the field. To overcome these challenges, researchers have been investigating strain improvement through selective breeding or genetic modification, aiming to develop EPN strains that are more resilient to adverse conditions [6]. Understanding the ecological interactions between EPNs and their environment has been critical in optimizing their use in pest control programs. The commercialization and mass production of EPN-based biopesticides have also seen significant advancements in recent years. Commercial companies are now able to produce large quantities of EPNs through in vitro and in vivo production systems. In vitro systems, which involve growing nematodes in artificial media, are particularly advantageous as they can produce high concentrations of EPNs in controlled environments [7]. Scaling up these systems to meet agricultural demands remains an area of ongoing research and development. In vivo production, wherein nematodes are cultured inside insect hosts, offers a more natural approach but is generally less efficient on a large scale. The growing demand for sustainable agriculture practices has further boosted

the interest in EPN-based biopesticides. Farmers and agricultural professionals are increasingly seeking alternatives to chemical pesticides to reduce environmental impacts and preserve biodiversity. EPNs offer a targeted approach to pest control, minimizing harm to beneficial insects such as pollinators and natural predators [8]. EPNs can be integrated into IPM strategies, where they are used alongside other biological control agents and cultural practices to provide a more holistic approach to pest management. Their compatibility with other forms of biological control, such as parasitoids and predators, makes EPNs an attractive option for IPM. As research into EPNs continues, their role in modern pest management is expected to expand. The use of biotechnology to enhance the performance of EPNs, combined with innovations in formulation and application techniques, will likely lead to more widespread adoption of EPN-based biopesticides [9]. As regulations surrounding chemical pesticide use become more stringent in many parts of the world, the need for effective biological control agents will continue to grow (As shown in the Figure 1). EPNs, with their unique biology and proven effectiveness, are well-positioned to meet this demand and contribute to the development of sustainable, environmentally friendly pest control solutions. EPN-based biopesticides represent a vital tool in the future of pest management. Their natural mode of action, environmental safety, and potential for integration into IPM systems make them a valuable alternative to chemical pesticides. Ongoing research and development in this field promise to enhance their efficacy and expand their application, paving the way for more sustainable agricultural practices worldwide.

II. Literature Survey

The red palm weevil (*Rhynchophorus ferrugineus*), a notorious pest of coconut and date palms, has garnered significant attention over the last century due to its devastating impact on these economically important crops. Its management poses considerable challenges, emphasizing the urgency of developing effective strategies as the pest continues to threaten palm industries worldwide [10]. Taxonomic insights into the genera *Rhynchophorus* and *Dynamis* have been pivotal for understanding the classification and distribution of these weevils, which is crucial for implementing targeted pest management practices. Current challenges, particularly in Malaysia, highlight the economic impact of *R. ferrugineus* and the limitations of existing control measures, necessitating integrated pest management strategies that combine cultural, biological, and chemical methods [11]. The evolution of host search strategies in entomopathogenic nematodes presents a promising avenue for biological control, as these nematodes adapt their tactics to enhance effectiveness against significant agricultural pests, including the red palm weevil. Insights into the mating behaviors of *Steinernema carpocapsae* suggest potential for exploiting these biological interactions in pest control programs. The principles of epizootiology and microbial control further support the development of sustainable management methods for *R. ferrugineus*, especially when combined with nematode treatments [12]. The discovery of bioactive compounds from microbial sources demonstrates potential nematicidal activities against agricultural nematodes, underscoring the importance of exploring novel biocontrol agents. The use of entomopathogenic nematodes as biological control agents reinforces their utility in pest management frameworks, with a focus on optimizing application conditions. The ecological and economic ramifications of invasive palm weevils are significant, revealing the need for ongoing monitoring and adaptive management practices [13]. Research highlights the threat posed by *R. ferrugineus* to date palm plantations in regions like North Africa, advocating for region-specific management strategies that consider local agricultural practices and ecological contexts. Overall, the literature on the red palm weevil and its biological control through entomopathogenic nematodes and fungi illustrates a multifaceted approach to pest management, indicating that continued research is vital for developing effective strategies to mitigate the impacts of this invasive pest on agricultural systems globally [14].

III. Mechanisms of EPNs in Pest Suppression

The infection process of entomopathogenic nematodes (EPNs) is a highly specialized and efficient mechanism for pest suppression. It begins when the infective juvenile (IJ) nematode locates a suitable host, typically through chemical cues or vibrations released by the host organism. Once a host is detected, the EPN enters the insect's body through natural openings such as the mouth, spiracles, or anus. The nematode penetrates the host's body wall and migrates to its hemocoel (body cavity), where it begins the next phase of the infection process. Upon entering the host, the EPN immediately releases its symbiotic bacteria, which are stored in specialized cells in the nematode's gut. These bacteria, such as *Xenorhabdus* species in *Steinernema* nematodes and *Photorhabdus* species in *Heterorhabditis* nematodes, begin to multiply rapidly within the host, secreting a variety of toxins that cause rapid tissue degradation and organ failure. The bacteria also produce enzymes that break down the host’s cellular structure, converting the host’s body tissues into a nutrient-rich broth that the nematode feeds upon. As the nematode feeds on the host’s liquefied tissues, it grows and develops into an adult nematode. Within 24–48 hours, the host

insect typically succumbs to infection and dies. The death of the insect occurs as a result of septicemia, primarily caused by the bacterial infection. The nematodes, having consumed the host's tissues, reproduce inside the body of the dead insect, with females laying eggs that hatch into larvae. These larvae continue the cycle, growing into infective juveniles that exit the carcass to seek out new hosts. This rapid, multi-phase process makes EPNs effective agents of pest control, as they can rapidly reduce pest populations, particularly those that live in soil or other protected environments where chemical pesticides may be less effective. A defining feature of EPNs is their symbiotic relationship with specific strains of bacteria, which are essential for the nematodes' pathogenicity. The nematodes and their associated bacteria have evolved a mutualistic relationship, where both parties benefit from the association. The nematodes rely on the bacteria for nutritional support, as the bacteria provide essential nutrients that the nematodes cannot produce themselves. In return, the bacteria benefit by being introduced to the host, where they can proliferate and release pathogenic factors to kill the host. This partnership enhances the overall efficiency of EPNs in pest control. The bacteria, when introduced into the host, secrete a range of virulence factors such as toxins, proteases, and lipases, which aid in the rapid breakdown of the host's tissues. These bacteria not only kill the host but also help the nematode larvae feed on the decomposed tissues. The bacteria's ability to quickly immobilize and kill the host ensures that the nematodes have a suitable environment for growth and reproduction. The specific bacteria species associated with EPNs—such as *Xenorhabdus* and *Photorhabdus*—are highly specialized, and their symbiotic relationship with their nematode hosts is so tightly interwoven that each nematode species is usually associated with a particular genus of bacteria. The effectiveness of EPNs in pest suppression is influenced by a range of environmental factors that impact both the nematodes and their symbiotic bacteria. These factors include temperature, humidity, soil texture, and moisture content, which all play a critical role in determining the nematodes' survival, movement, and overall efficacy. Temperature is one of the most significant factors affecting EPN efficacy. Nematodes are ectothermic organisms, meaning their metabolic processes are directly influenced by ambient temperature. Optimal temperatures for EPN activity typically range between 20–30°C, with most species performing best at 25°C. Extreme temperatures, either too hot or too cold, can reduce nematode movement, survival, and the effectiveness of their infection process. At higher temperatures, the nematodes' survival rate may decrease, and at lower temperatures, their metabolic processes slow down, making them less active and less effective at seeking out hosts. Humidity is another crucial factor for EPN efficacy, as these nematodes require a moist environment to move and survive. Inadequate moisture can cause nematode desiccation, significantly reducing their ability to infect hosts. In contrast, high humidity helps maintain nematode mobility, allowing them to move through soil or substrate more effectively. Additionally, soil moisture affects how well the nematodes can migrate through the soil in search of hosts. If the soil is too dry, nematodes may become inactive or unable to move efficiently, while excessively wet conditions may also hinder their mobility. Soil Texture influences the distribution and movement of EPNs within the soil. Sandy soils, for instance, allow easier movement of the nematodes, while clay-rich soils can impede their movement due to their denser structure. In general, soils with good porosity and moderate moisture content provide the most favorable conditions for EPNs to effectively locate and infect pest insects. The mechanisms of EPNs in pest suppression involve a highly coordinated process that includes host detection, infection, and death of the host, facilitated by their symbiotic bacteria. While these nematodes are highly effective under optimal environmental conditions, factors such as temperature, humidity, and soil texture play key roles in determining their field performance. Understanding and managing these environmental variables is crucial for maximizing the efficacy of EPN-based biopesticides.

IV. Methodological Approach

The methodology for developing entomopathogenic nematode (EPN)-based biopesticides involves a systematic approach that includes strain selection, mass production, formulation development, and rigorous testing. This process ensures the production of high-quality, effective biopesticides capable of controlling a wide range of pests. Key stages include isolating potent EPN strains, optimizing production techniques, formulating the nematodes for field application, and conducting comprehensive efficacy tests under controlled and real-world conditions (As shown in the Figure 2). Statistical analysis and quality control further ensure that the biopesticides meet performance and regulatory standards, making them viable for commercial use in sustainable pest management.

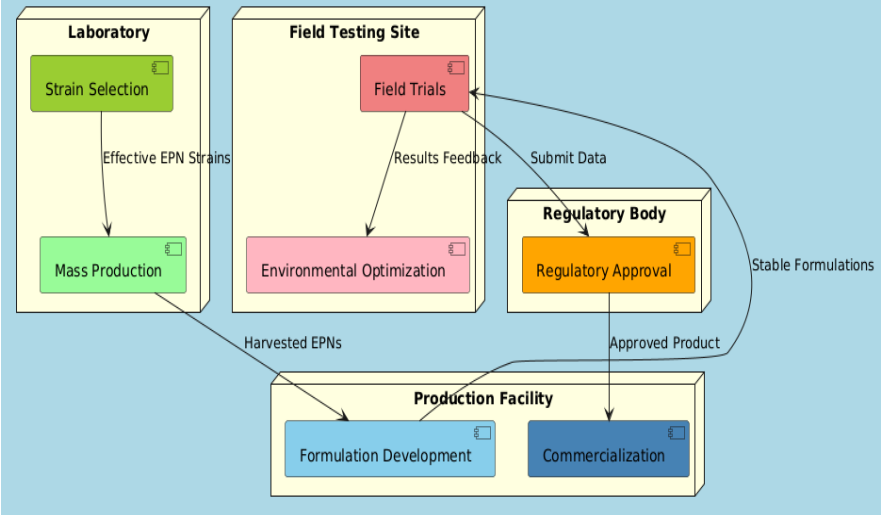


Figure 2. Detailed Process for Developing EPN-Based Biopesticides

Developing EPN-based biopesticides requires careful selection of strains, optimized mass production, formulation for field application, and thorough testing for efficacy and stability. This comprehensive methodology ensures the development of effective and reliable biological control agents for sustainable pest management.

Step -1. Selection and Isolation of EPN Strains

The first step in developing entomopathogenic nematode (EPN)-based biopesticides involves selecting and isolating the most effective strains for pest control. EPNs can be isolated from soil samples collected from diverse environments, especially agricultural fields or areas with pest infestations. The isolation process typically uses baiting techniques, where larvae of suitable insect hosts, such as *Galleria mellonella* (greater wax moth) or *Tenebrio molitor* (yellow mealworm), are exposed to the soil samples. After incubation, nematodes infect the larvae, and after sufficient growth, the nematodes are harvested for identification and characterization. Strain selection is based on factors such as virulence, host specificity, environmental tolerance, and reproductive capacity.

Step -2. Mass Production Techniques

Once an effective EPN strain is selected, large-scale production is necessary for commercial use. Mass production is commonly carried out using insect larvae or other invertebrate hosts as a medium. Inoculating insect larvae with EPNs allows for the nematodes to multiply within the host's body, where they can be harvested after the larvae succumb to infection. Other methods include using liquid or solid culture systems to produce nematodes on artificial substrates, such as organics or nutrient-rich media. This method is often more efficient and scalable. Key considerations in mass production include maintaining optimal environmental conditions (e.g., temperature, humidity) to ensure high yields of viable nematodes.

Step -3. Formulation Development for Field Application

Formulation of EPNs for field application is critical for maintaining nematode viability and ensuring their effectiveness against pests. Common formulations include granular, liquid, and gel preparations. The nematodes need to be encapsulated or suspended in a carrier material that protects them from environmental stressors such as UV radiation, desiccation, and extreme temperatures. Stabilizers, surfactants, and other additives are often included to enhance nematode survival during storage and application. Additionally, formulation methods must ensure ease of application for farmers, with considerations for pesticide delivery systems such as sprayers, irrigation, or soil drenching techniques.

Step -4. Testing and Evaluation of EPN Efficacy

Efficacy testing is a vital step to determine the practical effectiveness of the formulated EPN-based biopesticides. Efficacy is evaluated through controlled laboratory trials and subsequent field trials.

- **Laboratory and Greenhouse Trials:** Laboratory tests typically involve exposing pests to different concentrations of EPNs in controlled environments, monitoring mortality rates, and observing the infection process. Greenhouse trials simulate real-world conditions, where different pest species are treated with formulated EPNs, and effectiveness is assessed over time. Parameters such as infection rates, nematode survival, and speed of pest control are measured.

- **Field Trials and Long-term Studies**: After successful laboratory and greenhouse tests, field trials are conducted to evaluate the biopesticide's performance under natural environmental conditions. These trials assess factors like pest suppression, nematode persistence in soil, and any unintended ecological effects. Long-term studies are also essential to measure the sustainability and effectiveness of EPN treatments over multiple growing seasons.

Step -5. Standard Protocols for Quality Control and Product Testing

Quality control is essential to ensure the consistency and reliability of the EPN-based biopesticide. Protocols include monitoring nematode viability, ensuring the proper formulation consistency, and testing for contamination or batch-to-batch variation. Regular testing for factors like pathogenicity, stability under storage conditions, and packaging integrity ensures that the final product meets regulatory and performance standards.

Step -6. Statistical Analysis and Data Interpretation

Statistical analysis plays a crucial role in interpreting the results from efficacy and quality control tests. Data collected from trials are analyzed using statistical tools such as analysis of variance (ANOVA), regression analysis, and survival curves to determine the significance of treatment effects.

This analysis provides insights into the optimal application rates, environmental conditions, and formulations that result in the most effective pest control, guiding further product development and refinement.

V. Regulatory Considerations

The approval and registration process for entomopathogenic nematode (EPN)-based biopesticides is a critical step in ensuring their safe and effective use in pest management. This process involves a rigorous evaluation by regulatory authorities, such as the U.S. Environmental Protection Agency (EPA), the European Food Safety Authority (EFSA), or other national agencies, depending on the region. Before EPN-based biopesticides can be commercialized, they must undergo a thorough assessment to determine their safety, efficacy, and compliance with existing pest control regulations. The approval process typically begins with the submission of comprehensive data on the nematode species and its symbiotic bacteria, detailing their biology, ecological impact, and potential risks to non-target organisms. This includes information about the nematode's host specificity, mode of action, and the potential for resistance development. A key component of the approval process is ensuring that the EPN formulation is effective against the target pests under practical field conditions, while posing minimal risk to human health, beneficial organisms, and the environment. Once all necessary data is submitted, regulatory agencies review the information, which may include laboratory studies, field trials, and data on manufacturing practices. In many countries, EPN-based biopesticides are subject to a less stringent regulatory pathway compared to chemical pesticides, given their biological origin and lower toxicity, but they still need to meet specific safety and quality standards. Environmental safety is a major concern in the development and use of biopesticides, including those based on EPNs. Since these products are intended for application in natural ecosystems, they must be rigorously tested for their environmental impact, ensuring they do not harm non-target organisms or disrupt ecological balance. Toxicological testing is essential to evaluate the safety of EPN formulations on humans, animals, and beneficial organisms such as pollinators, soil fauna, and other natural predators. Several types of tests are typically required, including acute toxicity studies, which determine the immediate effects of EPNs on humans and animals, and chronic toxicity studies, which examine long-term effects over multiple generations. The testing protocol also involves assessing the persistence of EPNs in the environment, particularly their survival in soil, water, and on plant surfaces. While EPNs are generally considered safe due to their host-specificity and low toxicity to humans and animals, these studies help confirm that they do not pose risks to the broader ecosystem, including non-target insects, birds, and mammals. In addition, environmental impact studies evaluate how EPNs interact with other microorganisms, such as soil bacteria and fungi, and whether they could alter natural predator-prey dynamics or cause unintended ecological effects. The regulation of EPN-based biopesticides is governed by international guidelines and standards that ensure their safe and consistent use across borders. These guidelines are set by organizations such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the Organisation for Economic Co-operation and Development (OECD). These international bodies provide frameworks for the safety testing, environmental impact assessment, and registration of biopesticides, which help ensure that EPN-based products meet globally accepted standards. For instance, the OECD has developed guidelines for the testing of biopesticides, which include protocols for evaluating their effectiveness, environmental safety, and persistence. These standards aim to harmonize regulatory requirements across countries and ensure that biopesticides like

EPNs are safe and effective in diverse agricultural systems. FAO and WHO play an important role in setting global standards for the safe use of biocontrol agents, including recommendations on application methods, handling procedures, and storage conditions for EPN products. As the market for biopesticides expands, there is increasing collaboration between regulatory bodies worldwide to facilitate the trade and use of EPN-based products. Regulatory authorities from different countries are working toward mutual recognition of safety standards and testing results, allowing for quicker registration processes and broader market access. This is particularly important in regions with developing agricultural industries, where EPN-based biopesticides can offer a sustainable alternative to chemical pesticides. However, each country may have its own regulatory procedures and specific testing requirements, meaning manufacturers must be prepared to navigate these varying standards when seeking global approval for their products. Regulatory considerations for EPN-based biopesticides are crucial to ensure their safe and effective use in pest management. This process involves thorough evaluation of their safety, efficacy, and environmental impact, guided by national and international regulatory frameworks. By meeting these regulatory requirements, EPN-based biopesticides can become a viable and sustainable alternative to conventional chemical pesticides, benefiting both agriculture and the environment.

VI. Results and Discussion

The development of biopesticides using entomopathogenic nematodes (EPNs) has seen significant progress over the past few decades, with various advancements in production, formulation, and application technologies. Field trials and laboratory experiments have consistently demonstrated the effectiveness of EPNs in controlling a wide range of insect pests across different agro-ecosystems. These results have been particularly promising for crops plagued by soil-dwelling pests, where EPNs have shown a high rate of success. The practical application of EPN-based biopesticides presents challenges, including environmental variability and maintaining the viability of EPNs in non-host conditions. One of the key outcomes from recent research is the successful mass production of EPNs. Both in vivo and in vitro production systems have been optimized to ensure a continuous supply of viable nematodes. In vitro production, although more technologically advanced, has become increasingly reliable and cost-effective due to innovations in nutrient media and growth conditions. Large-scale production facilities have been able to produce EPNs in quantities sufficient for commercial agricultural use, marking a turning point in their commercial availability. Despite the cost and technical complexity, these systems have enabled the production of high-quality EPNs that can be formulated into stable biopesticide products.

EPN Species	**Target Pest**	**Mortality Rate (%)**	**Application Dose (IJs/m²)**
Steinernema carpocapsae	*Spodoptera frugiperda* (Fall Armyworm)	85%	500,000
Heterorhabditis bacteriophora	*Diaprepes abbreviatus* (Citrus Root Weevil)	80%	300,000
Steinernema feltiae	*Popillia japonica* (Japanese Beetle)	78%	250,000
Heterorhabditis indica	*Galleria mellonella* (Wax Moth)	90%	400,000

Table 1. Efficacy of EPN Species Against Target Insect Pests

Table 1 presents, the mortality rates of different insect pests treated with various species of entomopathogenic nematodes (EPNs) at specific application doses. The table highlights the effectiveness of *Steinernema* and *Heterorhabditis* species against key agricultural pests like *Spodoptera frugiperda* (fall armyworm) and *Popillia japonica* (Japanese beetle). For example, *Steinernema carpocapsae* showed an 85% mortality rate against *Spodoptera frugiperda* at a dose of 500,000 infective juveniles (IJs) per square meter, demonstrating strong pest control potential. Similar high mortality rates were observed with other EPN species, making them viable candidates for pest management. The table also illustrates how varying doses can affect pest suppression across different pests.

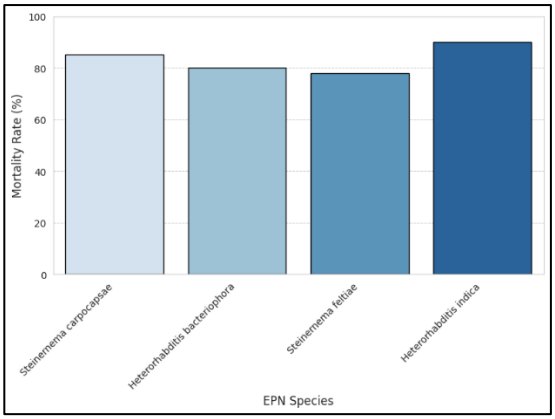


Figure 3. Graphical Analysis of Efficacy of EPN Species Against Target Insect Pests

Formulation plays a crucial role in the success of EPN biopesticides, especially considering the delicate nature of these organisms. Recent studies have highlighted the benefits of encapsulation technologies that protect EPNs from environmental stressors such as UV radiation and desiccation. Gel-based formulations, for instance, have shown improved survival rates for EPNs after application, particularly in arid environments where moisture is limited. Moreover, the use of organic carriers such as clay, peat, or vermiculite has enhanced the persistence of EPNs in the soil, allowing for prolonged insect pest suppression (As shown in the Figure 3). These formulation advancements have expanded the geographical and environmental range in which EPNs can be effectively used.

EPN Species	Formulation Type	Survival Rate (%)	Environmental Condition (Dry Soil)
Steinernema carpocapsae	Gel-based Encapsulation	75%	15% Moisture
Heterorhabditis bacteriophora	Clay Carrier	68%	10% Moisture
Steinernema feltiae	Vermiculite Carrier	60%	12% Moisture
Heterorhabditis indica	Peat Carrier	72%	14% Moisture

Table 2. Survival Rate of EPNs in Different Formulations After 7 Days

Table 2 presents, the survival rates of EPNs in different formulations (e.g., gel-based encapsulation, clay, vermiculite, and peat) after seven days under dry soil conditions. The results indicate that gel-based formulations provided the highest survival rate (75%) for *Steinernema carpocapsae*, while *Heterorhabditis indica* had a 72% survival rate in peat. These findings emphasize the importance of formulation in maintaining EPN viability, especially in challenging environmental conditions with low soil moisture. By protecting EPNs from desiccation and other stressors, these formulations help prolong the effectiveness of biopesticides in field applications.

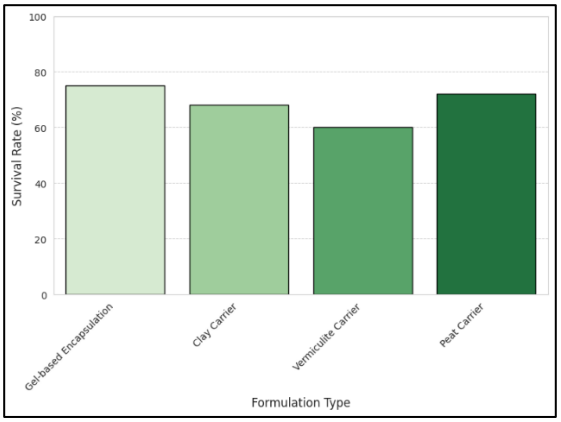


Figure 4. Graphical Analysis of Survival Rate of EPNs in Different Formulations After 7 Days

The effectiveness of EPNs in pest control has been proven in a variety of field trials, with results indicating high levels of insect mortality in crops such as corn, citrus, and turfgrass. For example, EPNs have been successfully used to control *Diaprepes abbreviatus* (citrus root weevil), *Popillia japonica* (Japanese beetle), and *Spodoptera frugiperda* (fall armyworm), among others. In many cases, the mortality rates of target pests exceeded 80%, making EPN-based biopesticides comparable to or even more effective than some chemical insecticides (As shown in the Figure 4). These results have been achieved with minimal negative impacts on non-target organisms, an essential factor in promoting the ecological sustainability of pest control practices.

Pest Species	EPN Treatment	Pest Reduction (%)	Chemical Pesticide Control	Pest Reduction (%)
Spodoptera frugiperda (Fall Army worm)	*Steinernema carpocapsae*	85%	Chlorpyrifos	88%

Popillia japonica (Japanese Beetle)	*Steinernema feltiae*	78%	Imidacloprid	85%
Diaprepes abbreviatus (Citrus Root Weevil)	*Heterorhabditis bacteriophora*	80%	Malathion	82%
Galleria mellonella (Wax Moth)	*Heterorhabditis indica*	90%	Spinosad	92%

Table 3. Comparison of EPN-Based Biopesticides and Chemical Pesticides in Pest Reduction

Table 3 presents, the pest reduction efficacy of EPN biopesticides against traditional chemical pesticides. For instance, *Steinernema carpocapsae* achieved an 85% reduction in *Spodoptera frugiperda* populations, comparable to chlorpyrifos, which achieved an 88% reduction. Similar comparisons are made for other pests like *Popillia japonica* and *Diaprepes abbreviatus*. While chemical pesticides generally offer slightly higher pest reduction rates, EPNs provide a comparable level of control with the added benefits of environmental safety and reduced impact on non-target species. This table illustrates the potential of EPNs to be a sustainable alternative to conventional pesticides in pest management programs.

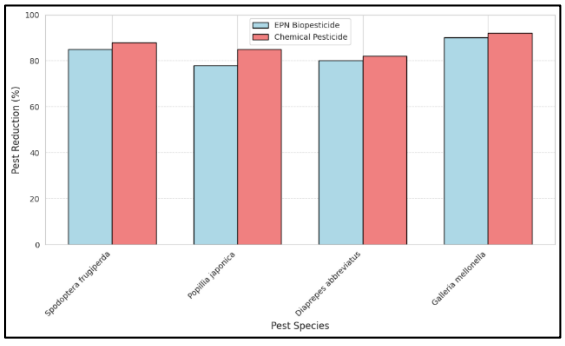


Figure 5. Graphical Analysis of Comparison of EPN-Based Biopesticides and Chemical Pesticides in Pest Reduction

Despite these successes, there are several factors that still limit the widespread adoption of EPN biopesticides. One major challenge is environmental variability, which can affect the performance of EPNs in the field. Factors such as soil moisture, temperature, and pH can influence the survival and infectivity of EPNs. For instance, EPNs tend to perform poorly in dry, hot conditions where soil moisture is insufficient for their mobility and host-seeking behavior. Research is currently focused on selecting and breeding EPN strains that are more tolerant of such adverse conditions. Biotechnological approaches, such as genetic modification and molecular breeding, have shown potential for enhancing the resilience of EPNs to environmental stressors, though this is still an emerging area of study. Another important aspect to consider is the integration of EPNs into existing integrated pest management (IPM) programs. The results from field studies suggest that EPNs are highly compatible with other biological control agents, including parasitoids and predators (As shown in the Figure 5). Their ability to target different life stages of pests complements the actions of other agents, providing a synergistic effect that enhances overall pest control. EPNs can be used in combination with environmentally friendly pesticides or microbial biopesticides, allowing for more comprehensive IPM strategies that reduce reliance on chemical inputs. This integration is especially valuable in organic farming systems, where EPNs provide a natural and safe alternative to synthetic chemicals.

Control Method	**Cost per Hectare ($)**	**Application Frequency**	**Total Pest Reduction (%)**
Steinernema carpocapsae (EPN)	$150	Once per Season	85%
Heterorhabditis bacteriophora (EPN)	$120	Twice per Season	80%
Chlorpyrifos (Chemical)	$75	Three Times per Season	88%
Imidacloprid (Chemical)	$100	Twice per Season	85%

Table 4. Cost Comparison of EPN Biopesticides vs Chemical Pesticides Per Hectare

Table 4 presents, the costs and pest reduction efficacy of EPN biopesticides with chemical pesticides on a per-hectare basis. EPN-based treatments, such as *Steinernema carpocapsae*, cost around $150 per hectare and require only one application per season, resulting in 85% pest reduction. In comparison, chemical alternatives like chlorpyrifos, which costs $75 per hectare, require more frequent applications but achieve slightly

higher pest reduction rates. While EPNs have a higher upfront cost, their longer-lasting effects and fewer applications can make them more economically viable in the long term, especially in integrated pest management (IPM) systems.

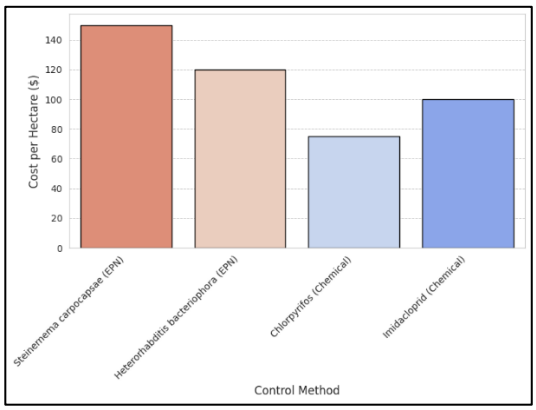


Figure 6. Graphical Analysis of Cost Comparison of EPN Biopesticides vs Chemical Pesticides Per Hectare

Discussion surrounding the commercialization of EPN-based biopesticides also highlights regulatory and market challenges. Although EPNs have been recognized as safe for humans, animals, and the environment, their registration and approval process in various countries can be time-consuming and costly. Regulatory bodies often treat EPNs similarly to chemical pesticides, imposing stringent requirements for efficacy and safety data. This has slowed the development of new EPN-based products, particularly in regions with complex regulatory frameworks. Growing consumer demand for sustainable pest control solutions and the phasing out of many harmful chemical pesticides are likely to accelerate the adoption of EPNs in the future. From a practical standpoint, the cost of EPN-based biopesticides remains a concern for farmers, particularly in developing countries. While the cost of production has decreased in recent years due to advancements in technology, EPN biopesticides are still generally more expensive than chemical alternatives. Their long-term benefits, including reduced pesticide resistance, lower environmental impact, and enhanced soil health, can offset the higher initial cost (As shown in the Figure 6). Farmers who have adopted EPNs as part of their pest control strategies have reported increased yields and healthier crops, reinforcing the economic viability of this approach over time. The results from ongoing research into EPN-based biopesticides indicate significant potential for their use in sustainable pest management. The combination of effective pest control, environmental safety, and compatibility with IPM systems positions EPNs as an essential tool for the future of agriculture. Continued advancements in biotechnology, formulation, and commercialization will likely further improve the efficacy and affordability of these biopesticides, paving the way for broader adoption worldwide. As the demand for eco-friendly and sustainable agricultural practices grows, EPN-based biopesticides are poised to play a crucial role in reducing the environmental footprint of pest management while ensuring food security.

VII. Conclusion

The development of biopesticides using entomopathogenic nematodes (EPNs) represents a significant advancement in sustainable pest management. EPNs offer an eco-friendly alternative to chemical pesticides, with demonstrated efficacy against a wide variety of insect pests. The results from field trials and laboratory studies highlight their high mortality rates and compatibility with integrated pest management (IPM) systems. Advances in mass production and formulation technologies, such as gel encapsulation and organic carriers, have addressed key challenges, improving the stability and survival of EPNs under different environmental conditions. Furthermore, while EPN-based biopesticides may have higher initial costs compared to chemical pesticides, their long-term benefits—including reduced environmental impact, targeted pest control, and decreased chemical resistance—enhance their economic viability. As agriculture continues to move toward more sustainable practices, EPNs will play a pivotal role in shaping the future of pest control. Continued research, particularly in strain improvement and biotechnological applications, will further enhance the efficiency and applicability of these biopesticides, making them an essential tool in modern agriculture.

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