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FORMULATION AND APPLICATION TECHNOLOGIES OF ENTOMOPATHOGENIC NEMATODES: FROM TRADITIONAL CARRIERS TO NANOFORMULATIONS

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ABSTRACT
Entomopathogenic nematodes (EPNs), primarily belonging to the families Steinernematidae and Heterorhabditidae, are widely recognized as effective and environmentally friendly biological control agents against a wide range of soil-dwelling and, increasingly, foliar insect pests. Although EPNs have a broad host range and can be integrated effectively into integrated pest management (IPM) programmes, their commercial use has been limited by several challenges, particularly the short shelf life of infective juveniles (IJs), their sensitivity to desiccation, and their susceptibility to ultraviolet (UV) radiation. Over the years, formulation technologies have evolved considerably, beginning with simple aqueous suspensions and moisture-retaining carriers such as sponge, vermiculite, peat, and clay. These approaches were subsequently followed by gel-based and alginate encapsulation systems and, later, by partially desiccated formulations such as wettable powders, water-dispersible granules, and wheat-gluten-based Pesta granules. More recent developments include superabsorbent polymer and hydrogel-based formulations, which can improve IJ survival under room-temperature storage conditions. Nanotechnology has further expanded the possibilities for EPN delivery, with materials such as titanium dioxide and silica nanoparticles being investigated as protective coatings, including their incorporation into Pickering emulsions to reduce desiccation and UV-related damage during foliar application. In addition, early research has explored the nanoformulation of EPN-derived venom proteins as potential bioactive insecticidal agents.
Alongside formulation development, application technologies have also progressed from conventional soil drenches to drip and micro-sprinkler irrigation, adjuvant-assisted foliar sprays, granular broadcasting, infected-host or cadaver-based delivery, and more precise encapsulated placement systems. The suitability and effectiveness of these application methods are closely linked to the characteristics of the formulation used. This review also considers the physiological mechanisms associated with desiccation and cold tolerance that influence formulation performance, as well as concerns related to nanoparticle biosafety, regulatory approval, and commercialization. Overall, the development of EPN formulations demonstrates a clear transition from simple carrier-based approaches toward engineered, stress-protective, and nanoscale delivery systems. Future advances in precision application, biodegradable polymers, hydrogels, and nanocarrier technologies may further improve EPN stability, field performance, and commercial viability, helping EPN-based biopesticides become more competitive with conventional insecticides.

KEYWORDS: Entomopathogenic nematodes, Steinernema, Heterorhabditis, , formulation, hydrogel, alginate, encapsulation, nanoparticles, , application technology, biological control

1. INTRODUCTION:

Entomopathogenic nematodes (EPNs), mainly belonging to the genera Steinernema and Heterorhabditis, are naturally occurring parasites of insects and are widely recognized as promising biological control agents for the management of economically important insect pests (Ramakuwela *et al*., 2025). These nematodes have attracted considerable attention because of their ability to infect and kill a broad range of insect hosts, their relative safety to plants and vertebrates, and their compatibility with integrated pest management (IPM) programmes(Abdel Gawad *et al*., 2023). Unlike many conventional insecticides, EPNs represent a living biological control system in which the nematode and its associated bacterial symbiont work together to overcome and kill the insect host (Tarasco *et al*., 2023).
The insecticidal activity of EPNs is closely associated with their symbiotic bacteria. Steinernema species harbour bacteria belonging mainly to the genus Xenorhabdus, whereas Heterorhabditis species are associated with bacteria of the genus Photorhabdus. The infective juveniles (IJs), which are developmentally arrested third-stage juveniles, carry these bacteria and actively search for suitable insect hosts (Burnell & Stock *et al*., 2000). Once an IJ encounters a susceptible insect, it enters the host through natural openings such as the mouth, anus, or spiracles, or in some cases through the cuticle. After entering the haemocoel, the nematode releases its symbiotic bacteria (Griffin *et al*., 2005). The bacteria multiply rapidly within the insect haemolymph and produce a combination of toxins, enzymes, and other virulence factors that disrupt host physiology and lead to death, primarily through

septicemia (Chattopadhyay *et al*., 2004).The nematodes subsequently feed on the bacterial biomass and degraded host tissues, allowing them to complete their development and reproduce within the cadaver(Griffin *et al*., 2005). The infective juvenile is particularly important from a biological control perspective because it is the only free-living stage that actively searches for and infects a new host (Askary *et al*., 2018). IJs can survive outside the insect host for varying periods and respond to environmental and chemical cues associated with potential prey (Lortkipanidze *et al*., 2016). Their host-searching behaviour may involve movement toward carbon dioxide, insect-derived volatiles, and volatile organic compounds released by plants following herbivore attack. Their ability to move through soil pores and locate concealed insect stages makes EPNs especially useful against soil-dwelling pests, including larvae, pupae, and other vulnerable developmental stages (Gozel, U., & Gozel, C., 2016). Some EPN species have also demonstrated potential against insects inhabiting above-ground plant parts, although foliar application presents additional challenges because of exposure to ultraviolet (UV) radiation and rapid water loss (Platt *et al*., 2020).

Another important advantage of EPNs is their relatively flexible production system. They can be multiplied in living insect hosts or produced on artificial media using in vitro liquid or solid fermentation techniques (Nagesh *et al*.,2017). Advances in mass-production technology have improved the availability of EPNs for agricultural and horticultural applications (Ramakuwela *et al*., 2025). Their biological activity can also be combined with several IPM practices, including compatible chemical pesticides, microbial biocontrol agents, cultural practices, and other environmentally based pest management approaches (Zhou *et al*.,2024). This compatibility has contributed to increasing interest in EPNs as alternatives or complements to conventional insecticides (Kaur *et al*., 2026).

One of the major limitations is the difficulty of maintaining the viability and infectivity of IJs during storage, transportation, and field application (Koppenhöfer., 2007). EPNs are living organisms and are therefore considerably more sensitive to environmental conditions than conventional chemical pesticides (Matuska-Łyżwa *et al*., 2024). Factors such as desiccation, temperature fluctuations, UV radiation, soil moisture, soil texture, and unsuitable application conditions can substantially reduce their survival and effectiveness (Kaya ., 1990)

Formulation and delivery therefore represent two of the most important factors determining the practical success of EPN-based biopesticides (Cruz-Martínez *et al*., 2017). Early formulations relied largely on aqueous suspensions ,sponge, vermiculite, peat, clay, gels, alginate, polymers, and other carrier systems (Askary & Ahmad., 2017). More advanced formulations have attempted to protect IJs from environmental stress while extending their shelf life and facilitating their application using conventional agricultural equipment (Fatimah *et al*., 2025). Similarly, application methods have expanded from simple soil drenches to irrigation-based delivery, granular formulations, foliar sprays, infected-host applications, and more targeted placement techniques (Chacon Hurtado., 2026).

Thus, although EPNs possess several characteristics that make them attractive alternatives to conventional insecticides, their successful commercialization depends not only on nematode virulence and host-searching ability but also on the development of stable formulations and efficient delivery systems(Fatimah *et al*., 2025). The continuing challenge is to maintain high IJ survival and infectivity from production and storage through transportation and application until the nematodes reach their target insect (Grewal., 2002). Improvements in formulation science, polymer technology, controlled-release systems, and precision application may therefore play a decisive role in expanding EPN use from small-scale and specialized applications to wider commercial pest management programmes (Raypuriya *et al*., 2025). Understanding the relationship between EPN biology, formulation characteristics, environmental conditions, and application technology is consequently essential for developing reliable and economically viable EPN-based biopesticides (Kaur *et al*., 2026).

2. Mass Production as the Foundation of Formulation

Before formulation development, EPNs need to be produced in sufficient quantities, and the production method can strongly influence the types of formulations that are practical for subsequent use (Askary & Ahmad., 2017). Three major production approaches are currently used: in vivo production, in which nematodes are multiplied within living insect hosts, most commonly *Galleria mellonella* larvae; in vitro solid-media culture; and in vitro liquid fermentation **(**Han *et al*., 2024). Among these methods, in vivo production requires relatively low initial investment but has limited scalability because labour, equipment, and insect-rearing requirements increase with production volume. In contrast, in vitro liquid fermentation requires greater initial investment in infrastructure and equipment but generally provides better economic efficiency at large production scales and is therefore widely used for commercial EPN production (Dunn *et al*.,2021). In vivo production remains particularly useful for laboratory studies, greenhouse experiments, small-scale field trials, and niche applications where large-scale production facilities are not available (Sikora & Schuster .,1998).

For commercial liquid fermentation, EPN production generally starts in Erlenmeyer flasks, where adequate aeration and agitation support nematode development, before the culture is gradually transferred to larger systems (Cortés-Martínez & Chavarría-Hernández .,2020). The composition of the liquid culture medium is an important factor influencing both nematode development and the growth of their symbiotic bacteria (Cho *et al*.,2011). Several studies have also shown that production efficiency can be affected by parameters such as inoculum age, medium viscosity, flask size, and aeration rate, which influence IJ recovery and yield in liquid, solid, and biphasic culture systems (Leite *et al*.,2017).

3. Formulation Categories

3.1 Aqueous and Carrier-Based Formulations

Direct aqueous suspension is one of the simplest ways to formulate EPNs; however, its limited stability has encouraged the development of alternative carrier-based formulations (Nxitywa & Malan., 2021). Over the years, several materials have been used as EPN carriers, including clay, activated charcoal, sponge, vermiculite, peat, alginate gels, and water-dispersible granules. These formulations can be applied using conventional agricultural spraying equipment, irrigation systems, or injection methods (Askary & Ahmad., 2017). The shelf life of many of these formulations can be improved by lowering nematode metabolic activity through partial desiccation and/or storage at low temperatures, generally around 4–15 °C (Shapiro-Ilan *et al*., 2001). However, EPNs have a limited ability to withstand complete dehydration, and inappropriate drying conditions or excessive rates of desiccation have resulted in complete mortality in some *Steinernema* studies(Ramakrishnan *et al*., 2024).

3.2 Alginate Encapsulation

Alginate-based encapsulation has a well-established history in the development of EPN formulations. Alginate is a polysaccharide obtained from brown algae and was first used for EPN encapsulation by Kaya and Nelsen in 1985 (Kim *et al*.,2015). In their approach, *Heterorhabditis heliothidis* and *Steinernema feltiae* were immobilized by adding an EPN-containing alginate solution dropwise into calcium chloride, resulting in the formation of alginate beads (kim *et al*., 2021) . These alginate hydrogels provide a moist environment and offer some protection against UV radiation, thereby helping to reduce the problems associated with the short shelf life and sensitivity of EPNs to desiccation and UV exposure (Colin *et al*., 2024). However, a major limitation reported in several studies is the tendency of infective juveniles (IJs) to escape from conventional alginate beads within a few days, particularly when the formulations are stored without refrigeration. This rapid release is considered a challenge for their commercial use (Kagimu & Malan., 2019).

To overcome this limitation, subsequent studies developed capsules containing a liquid core. In this method, EPNs were incorporated into a mixture of calcium salts of lactic and gluconic acids, which was then introduced into an alginate solution to form the capsules (Darsouei *et al*.,2025). These formulations showed promising field performance, including protection of maize against Western corn rootworm damage (Jaffuel *et al*.,2019). Further improvements involved modifying the capsule-forming temperature and applying an additional treatment with excess calcium ions. These modifications produced capsules with stronger outer shells and improved nematode retention by approximately 40% (Kim *et al*., 2015).

In a detailed study involving three EPN species, calcium alginate formulations were able to maintain IJ survival for up to three months at room temperature (Darsouei *et al*.,2025). However, survival varied among species and temperatures. *Heterorhabditis bacteriophora* showed better survival at 25 °C, whereas *Steinernema carpocapsae* performed better at 8 °C (Bütüner *et al*., 2023). In contrast, the survival of *S. feltiae* was not significantly affected by temperature(Darsouei *et al*.,2025). Importantly, the pathogenicity of the nematodes remained stable throughout the storage period (Ramakuwela *et al*.,2015). Field studies have also demonstrated that alginate-encapsulated EPNs can provide control comparable to that achieved with aqueous EPN sprays against target larvae such as *Diabrotica balteata* and can help protect crops (Darwish *et al*., 2025). However, successful control depended strongly on application timing, with treatments being more effective when applied before substantial pest damage occurred. This demonstrates that an effective formulation alone cannot compensate for inappropriate application timing.

Further formulation studies, including those conducted using South African EPN isolates, reported that approximately 10–20% of IJs escaped from alginate beads depending on storage temperature (Kagimu & Malan., 2019).. These findings highlight an important balance between providing sufficient protection to the nematodes and allowing their controlled release from the encapsulation system.

3.3 Wettable Powders, Water-Dispersible Granules, and Pesta Granules

Dry and partially desiccated formulations were developed mainly to overcome the limited stability of EPNs at room temperature (Askary & Ahmad., 2017). Among these, water-dispersible granules (WDGs) have been widely investigated and used as inert carrier-based systems designed to maintain nematode quality during storage and transportation (Fadiji *et al*., 2024). However, their effectiveness and storage performance can vary considerably between species of *Steinernema* and *Heterorhabditis* (Li *et al*., 2026). Another important development was the "Pesta" granule, a dry, wheat-gluten-based formulation originally developed for *Steinernema carpocapsae*. This formulation represented an important step toward extending EPN shelf life compared with earlier granular formulations. Subsequent studies further modified and optimized Pesta granules to improve their storage stability and maintain nematode viability (Connick *et al*., 1993).

3.4 Polymer, Superabsorbent, and Hydrogel Formulations

Superabsorbent polymer technology has been developed as a practical approach to improving the room-temperature shelf life of EPN formulations. In a controlled six-month factorial study, *Steinernema carpocapsae* was formulated using carboxymethyl cellulose (CMC) supplemented with a superabsorbent polymer and an antimicrobial agent. The results showed a marked improvement in nematode survival. In formulations without antimicrobial treatment, survival declined to ≤0.2% by the second month. In contrast, the optimized formulation containing 4% superabsorbent polymer and sorbic acid maintained considerably higher nematode survival at room temperature throughout the six-month storage period, without any detectable reduction in virulence against test larvae (Sharghi *et al*., 2025).

In another study, Barricade® Fire Gel and potassium polyacrylate hydrogel formulations were compared using several EPN species produced through both in vivo and in vitro methods (Zulu *et al*., 2025).

3.5 Nanotechnology-Based Formulations

Nanotechnology-based approaches represent a newer direction in EPN formulation, with much of the current research focusing on protecting the nematode cuticle from two major environmental stresses encountered under field conditions: desiccation and ultraviolet (UV) radiation **(**Guleria *et al*., 2026). Nanotechnology, controlled-release systems, automation, and drones are improving EPN-based pest control. Nanotechnology enhances EPN stability, survival, and field efficacy, enabling more precise and efficient pest management (Neelam Thakur *et al*, 2025).

Kotliarevski *et al*. (2022) developed an eco-friendly EPN formulation using titanium dioxide nanoparticles (TiO_2-NH_2) and mineral oil in Pickering emulsions. Oil–water ratios of 40:60 and 50:50 provided optimal droplet size and compatibility with nematodes. The formulation maintained EPN viability for up to 120 days and preserved infectivity for 150 days, demonstrating high stability and effectiveness.Nano-carriers enhance EPN formulations by protecting them from desiccation, UV radiation, and temperature stress while enabling controlled release and improved delivery (Cruz-Martínez *et al*., 2017). They extend shelf life and synchronize nematode release with pest activity, improving reliability in biocontrol.Wu *et al*. (2023) showed that TiO_2-NH_2 nanoparticles effectively protect *Steinernema carpocapsae,* from UV, maintaining survival and virulence.

Further development of this approach showed that Pickering emulsion-based coatings could improve the survival of IJs on exposed plant foliage. This is particularly important because foliar application has traditionally been challenging for EPNs due to rapid water loss and UV exposure. Such formulations therefore provide a potential approach for extending the use of EPNs in the management of above-ground insect pests (Ramakrishnan *et al*., 2023). In related research, silica-NH_2 nanoparticle-based emulsions have also been investigated for their ability to protect EPNs from UV radiation. Their performance was compared with Barricade® gel and aqueous formulations under different periods of UV exposure (Kotliarevski *et al*., 2022)

In addition to protecting whole nematodes, a different and emerging nanotechnology approach involves formulating isolated EPN venom proteins into nanoparticles for use as independent insecticidal agents. This strategy separates the active insecticidal component from the living nematode itself and represents a potentially different route for exploiting EPN-derived bioactive molecules (Kaur *et al*., 2026). However, this area is still at an early proof-of-concept stage and requires further research before practical agricultural application can be considered.

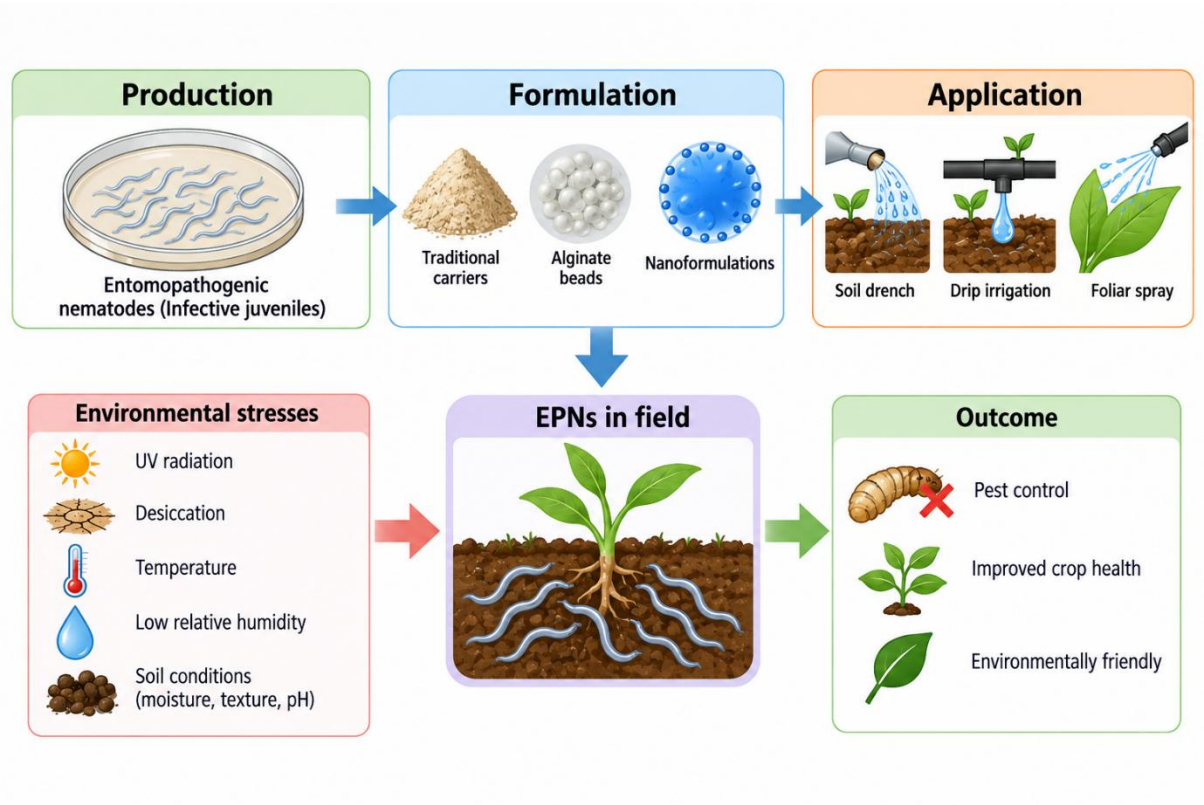


Figure 1: Overview of EPN production, formulation, application technologies, environmental stresses, and field outcomes.

4. Methods of Application

4.1 Cadaver-Based ("Infected Host") Application

Applying EPNs through infected insect cadavers, rather than releasing free infective juveniles (IJs) in aqueous suspension, has shown promising results in several application studies (Cruz-Martínez *et al*., 2017). In a controlled

turfgrass study targeting *Hoplia philanthus*, cadavers of *Heterorhabditis bacteriophora* coated with a kaolin-starch mixture provided considerably better control than aqueous EPN applications (Ansari *et al*., 2009). Freshly applied cadavers resulted in 62% control under greenhouse conditions, compared with 39–43% obtained with aqueous suspensions. Under field conditions, the advantage of the cadaver-based approach was even more evident after one year, with cadaver applications maintaining more than 90% control, whereas aqueous applications of the same nematode provided only about 55% control (Dolinski *et al*., 2015). The kaolin-starch coating also provided better protection and preservation of the infected cadavers than untreated cadavers(Ansari *et al*., 2009).

However, cadaver-based delivery does not always provide better pest control, as its effectiveness can depend strongly on the target pest and environmental conditions(Dolinski *et al*., 2015). For example, studies conducted against wireworms found that *Steinernema carpocapsae* and *S. riobrave* applied through infected cadavers caused 50–68% mortality under greenhouse conditions, but none of the four EPN strains tested provided effective control under field conditions (Sandhi *et al*., 2021). This highlights the importance of considering the specific pest, environment, and application conditions when evaluating cadaver-based EPN delivery.

The physical condition of the cadaver is also an important factor affecting its performance. Studies that simulated damage caused by scavengers showed that damage to the cadaver cuticle significantly reduced both the number and size of IJs emerging from the cadaver. This reduction was particularly associated with increased moisture loss under dry conditions. Therefore, protecting infected cadavers with suitable coatings or formulations can help maintain their structural integrity, reduce moisture loss, and support the successful emergence of IJs in the field (Cassells *et al*., 2024). These findings indicate that the effectiveness of cadaver-based delivery depends not only on the EPN species and target pest but also on how well the infected cadaver is protected during storage and after field application.

4.2 Precision and Encapsulated Delivery

Precision application technologies have also been explored to improve the placement and delivery of EPNs in agricultural soils (Erdoğan *et al*., 2023). One such approach uses **gelatin capsules** to deliver EPNs at specific locations through precision-planting equipment. Field trials using this method resulted in approximately **53–67% larval mortality**, with control levels statistically comparable to those achieved through conventional drip-irrigation application(Thakur *et al*., 2025). This suggests that encapsulated delivery can provide effective pest suppression while allowing EPNs to be placed more precisely in the crop environment.

Studies examining nematode dispersal further showed that the use of gelatin capsules did not negatively affect the movement of EPNs through the soil when compared with conventional application methods. Once the capsule releases its contents, the IJs can disperse through the surrounding soil and reach potential insect hosts. Such targeted placement may also help reduce unnecessary exposure of EPNs to unfavourable environmental conditions and improve the efficiency of nematode use by concentrating them closer to areas where susceptible pests are likely to occur (Ulu & Erdoğan., 2023). Therefore, precision capsule-based delivery represents a promising approach for integrating EPNs with modern agricultural application technologies, particularly where accurate placement and efficient use of nematode inoculum are important.

4.3 Irrigation-Based Delivery

Drip irrigation has been evaluated as a method for delivering EPNs, and studies have shown that injecting EPN suspensions into drip lines can result in relatively uniform nematode distribution along the irrigation line. However, the percentage of nematodes recovered from the drip emitters varied considerably, ranging from 42% to 92%, depending on the conditions (Lee-Park *et al*., 2026).

In citrus applications, micro-sprinkler irrigation systems have been shown to distribute EPNs more uniformly than drip-irrigation systems. This difference was particularly associated with the lower flow rates of drip systems, which can result in less consistent nematode deposition along the irrigation lines throughout the orchard (Ulu & Erdoğan., 2023).

Table 1: Different formulation carries, its active form and target pests.

Formulation Carrier	**Active Formulation Matrix**	**Target Pests & Performance**	**Primary Operational Limitations**
Aqueous Suspensions	Water carrier with mild surfactants (Kaur *et al*.,2026)	Broad foliar and soil pests (Raypuriya *et al*.,2025)	High UV susceptibility and strict refrigeration requirements (Kaur *et al*.,2026)

Formulation Carrier	Active Formulation Matrix	Target Pests & Performance	Primary Operational Limitations
Infected Cadavers	Kaolin-starch protective coatings (Kim *et al*.,2021)	Soil grubs; >90% control over 1 year (Ansari *et al*.,2009)	Fragile handling and mechanical application bottlenecks (Kim *et al*.,2021)
Water-Dispersible Granules	Silica, clay, and starch blends (Kim *et al*.,2021)	Soil rootworms; storage up to 180 days (Cruz-Martinez *et al*.,2017)	Variable rehydration kinetics and carrier residue costs (Kaur *et al*.,2026)
Alginate Hydrogels	Ca^{2+}-crosslinked alginate with glycerol (Kim *et al*.,2021)	Western corn rootworm; equal to aqueous sprays (Kim *et al*.,2021)	Slow juvenile release rates without free moisture (Kim *et al*.,2021)
Protective Spray Gels	Carboxymethyl cellulose & vegetable oils (Waweru *et al*.,2025)	Fall armyworm; matched pyrethroid efficacy (Fallet *et al*.,2022)	Deposition friction and viscosity in standard sprayers (Darsouei *et al*.,2025)

CONCLUSION:

Entomopathogenic nematodes (EPNs) have considerable potential as environmentally compatible biological control agents, but their wider use is limited by the sensitivity of infective juveniles to desiccation, UV radiation, temperature, and other environmental stresses. Over the years, formulation technologies have progressed from simple aqueous suspensions and traditional carriers to alginate encapsulation, dry formulations, hydrogels, superabsorbent polymers, and more recently, nanoparticle-based systems. Similarly, application methods have developed from conventional soil drenches to irrigation-based delivery, foliar sprays, infected-host cadavers, and precision placement.

Recent nanoformulation approaches, particularly nanoparticle coatings and Pickering emulsions, offer promising possibilities for improving IJ protection and extending their use under challenging field conditions. However, issues related to production cost, formulation stability, environmental safety, scalability, and regulatory requirements still need to be addressed. Future research should focus on developing cost-effective, biodegradable, and stable formulations combined with efficient precision-delivery methods. Continued collaboration between researchers and industry will be essential for translating these technological advances into reliable and commercially viable EPN-based biopesticides.

REFERENCES:

1. Ramakuwela, T., Tarasco, E., Chavarría-Hernández, N., & Toepfer, S. (2025). Entomopathogenic nematodes: Commercial use and future perspectives. Journal of Invertebrate Pathology, 212, 108388. https://doi.org/10.1016/j.jip.2025.108388

2. Abdel Gawad, M. M., Ruan, W., & Hammam, M. (2023). Entomopathogenic nematodes: Integrated pest management and new vistas. Egyptian Journal of Agronematology, 22(1), 1-18. https://doi.org/10.21608/ejaj.2023.280551

3. Tarasco, E., Fanelli, E., Salvemini, C., El-Khoury, Y., Troccoli, A., Vovlas, A., & De Luca, F. (2023). Entomopathogenic nematodes and their symbiotic bacteria: from genes to field uses. Frontiers in insect science, 3, 1195254. https://doi.org/10.3389/finsc.2023.1195254

4. Burnell, A., & Stock, S. P. (2000). Heterorhabditis, Steinernema and their bacterial symbionts-lethal pathogens of insects. Nematology, 2(1), 31-42. https://doi.org/10.1163/156854100508872

5. Griffin, C. T., Boemare, N. E., & Lewis, E. E. (2005). Biology and behaviour. In Nematodes as biocontrol agents (pp. 47-64). Wallingford UK: CABI publishing. https://doi.org/10.1079/9780851990170.0047

6. Chattopadhyay, A., Bhatnagar, N. B., & Bhatnagar, R. (2004). Bacterial insecticidal toxins. Critical reviews in microbiology, 30(1), 33-54. https://doi.org/10.1080/10408410490270712

7. Askary, T. H., Jamal Ahmad, M., Wani, A. R., Mohiddin, S., & Sofi, M. A. (2018). Behavioural ecology of entomopathogenic nematodes, Steinernema and Heterorhabditis for insect biocontrol. In Sustainable agriculture reviews 31: Biocontrol (pp. 425-441). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-94232-2_8

8. Lortkipanidze, M. A., Gorgadze, O. A., Kajaia, G. S., Gratiashvili, N. G., & Kuchava, M. A. (2016). Foraging behavior and virulence of some entomopathogenic nematodes. Annals of Agrarian Science, 14(2), 99-103. https://doi.org/10.1016/j.aasci.2016.05.009

9. Gozel, U., & Gozel, C. (2016). Entomopathogenic nematodes in pest management. Integrated pest management (IPM): environmentally sound pest management, 55. https://doi.org/10.5772/63894

10. Platt, T., Stokwe, N. F., & Malan, A. P. (2020). A review of the potential use of entomopathogenic nematodes to control above-ground insect pests in South Africa. South African Journal of Enology and Viticulture, 41(1), 1-16.
https://doi.org/10.21548/41-1-2424

11. Nagesh, M., Askary, T. H., Balachander Manohar, B. M., Arakalagud, S. N., & Rajan, R. (2017). Strategies for making entomopathogenic nematodes cost-effective biocontrol agents. In Biocontrol agents: entomopathogenic and slug parasitic nematodes (pp. 596-619). Wallingford UK: CABI. https://doi.org/10.1079/9781786390004.0596

12. Zhou, W., Arcot, Y., Medina, R. F., Bernal, J., Cisneros-Zevallos, L., & Akbulut, M. E. (2024). Integrated pest management: an update on the sustainability approach to crop protection. ACS omega, 9(40), 41130-41147. https://doi.org/10.1021/acsomega.4c06628

13. Kaur, A., Sirengo, D. K., Karki, P., Powers, T. O., & Brown, A. (2026). Harnessing entomopathogenic nematodes for sustainable pest management: mechanisms, challenges, and innovations. Frontiers in Plant Science, 17, 1755114. https://doi.org/10.3389/fpls.2026.1755114

14. Koppenhöfer, A. M. (2007). Nematodes. In Field manual of techniques in invertebrate pathology: application and evaluation of pathogens for control of insects and other invertebrate pests (pp. 249-264). Dordrecht: Springer Netherlands.
https://doi.org/10.1007/978-1-4020-5933-9_11

15. Matuska-Łyżwa, J., Duda, S., Nowak, D., & Kaca, W. (2024). Impact of abiotic and biotic environmental conditions on the development and infectivity of entomopathogenic nematodes in agricultural soils. Insects, 15(6), 421. https://doi.org/10.3390/insects15060421

16. Kaya, H. (1990). 5. Soil Ecology. Entomopathogenic nematodes in biological control, 93.

17. Cruz-Martínez, H., Ruiz-Vega, J., Matadamas-Ortíz, P. T., Cortés-Martínez, C. I., & Rosas-Diaz, J. (2017). Formulation of entomopathogenic nematodes for crop pest control-a review. Plant Protection Science, 53(1), 15. https://doi.org/10.17221/35/2016-PPS

18. Askary, T. H., & Ahmad, M. J. (2017). Entomopathogenic nematodes: mass production, formulation and application. In Biocontrol agents: entomopathogenic and slug parasitic nematodes (pp. 261-286). Wallingford UK: CABI. https://doi.org/10.1079/9781786390004.0261

19. Fatimah, N., Askary, T. H., & Abd-Elgawad, M. M. (2025). Factors influencing the performance of entomopathogenic nematodes: from laboratory to field conditions. Egyptian Journal of Biological Pest Control, 35(1), 29. https://doi.org/10.1186/s41938-026-00902-6 https://doi.org/10.1186/s41938-025-00864-1

20. Chacon Hurtado, J. A. (2026). Entomopathogenic Nematodes and Volatile Organic Compounds to Control Wireworms.

21. Grewal, P. S. (2002). Formulation and application technology. In Entomopathogenic nematology (pp. 265-287). Wallingford UK: CABI publishing. https://doi.org/10.1079/9780851995670.0265

22. Raypuriya, N., Ghanghas, R., Das, A., Priyadarshini, P., Yadav, S., Dose, J. G., ... & Maheshwari, S. (2025). Advances in Biological Control of Crop Pests through Entomopathogenic Nematodes: A Review. Uttar Pradesh Journal of Zoology, 46(17), 8-22. https://doi.org/10.56557/upjoz/2025/v46i175205

23. Han, R., Leite, L. G., Yan, X., Chacon-Orozco, J. G., & Shapiro-Ilan, D. I. (2024). Mass production. In Entomopathogenic nematodes as biological control agents (pp. 182-200). GB: CABI. https://doi.org/10.1080/00207543.2023.2255681 https://doi.org/10.1016/j.ijpe.2024.109340 https://doi.org/10.1007/s11740-023-01241-3 https://doi.org/10.1080/00207543.2023.2236241

24. Dunn, M. D., Belur, P. D., & Malan, A. P. (2021). A review of the in vitro liquid mass culture of entomopathogenic nematodes. Biocontrol Science and Technology, 31(1), 1-21. https://doi.org/10.1080/09583157.2020.1837072

25. Sikora, R. A., & Schuster, R. P. (1998, November). Novel approaches to nematode IPM. In Mobilizing IPM for sustainable banana production in Africa: proceedings of a meeting in Nelspruit, South Africa (pp. 127-136).

26. Cortés-Martínez, C. I., & Chavarría-Hernández, N. (2020). Production of entomopathogenic nematodes in submerged monoxenic culture: a review. Biotechnology and Bioengineering, 117(12), 3968-3985. https://doi.org/10.1002/bit.27515

27. Cho, C. H., Whang, K. S., Gaugler, R., & Yoo, S. K. (2011). Submerged monoxenic culture medium development for Heterorhabditis bacteriophora and its symbiotic bacterium Photorhabdus luminescens: protein sources. Journal of Microbiology and Biotechnology, 21(8), 869-873. https://doi.org/10.4014/jmb.1010.10055

28. Leite, L. G., Shapiro-Ilan, D. I., Hazir, S., & Jackson, M. A. (2017). Effect of inoculum age and physical parameters on in vitro culture of the entomopathogenic nematode Steinernema feltiae. Journal of Helminthology, 91(6), 686-695. https://doi.org/10.1017/S0022149X16000821

29. Nxitywa, A., & Malan, A. P. (2021). Formulation of entomopathogenic nematodes for the control of key pests of grapevine: A review. South African Journal of Enology and Viticulture, 42(2), 123-135. https://doi.org/10.21548/42-2-4479

30. Shapiro-Ilan, D. I., Lewis, E. E., Behle, R. W., & McGuire, M. R. (2001). Formulation of entomopathogenic nematode-infected cadavers. Journal of invertebrate pathology, 78(1), 17-23. https://doi.org/10.1006/jipa.2001.5030

31. Ramakrishnan, J., Ment, D., & Glazer, I. (2024). Survival mechanisms of entomopathogenic nematodes: current knowledge and its integration toward a better performing biological control agent. Entomopathogenic Nematodes as Biological Control Agents, 95-111. https://doi.org/10.1079/9781800620322.0005

32. Kim, J., Jaffuel, G., & Turlings, T. C. (2015). Enhanced alginate capsule properties as a formulation of entomopathogenic nematodes. BioControl, 60(4), 527-535. https://doi.org/10.1007/s10526-014-9638-z

33. Kim, J., Hiltpold, I., Jaffuel, G., Sbaiti, I., Hibbard, B. E., & Turlings, T. C. (2021). Calcium-alginate beads as a formulation for the application of entomopathogenic nematodes to control rootworms. Journal of Pest Science, 94(4), 1197-1208. https://doi.org/10.1007/s10340-021-01349-4

34. Colin, C., Akpo, E., Perrin, A., Cornu, D., & Cambedouzou, J. (2024). Encapsulation in alginates hydrogels and controlled release: An overview. Molecules, 29(11), 2515. https://doi.org/10.3390/molecules29112515

35. Kagimu, N., & Malan, A. P. (2019). Formulation of South African entomopathogenic nematodes using alginate beads and diatomaceous earth: N. Kagimu, AP Malan. BioControl, 64(4), 413-422. https://doi.org/10.1007/s10526-019-09945-1

36. Darsouei, R., Karimi, J., & Stelinski, L. L. (2025). Properties of enhanced calcium-alginate beads as a formulation for disseminating the entomopathogenic nematodes Heterorhabditis bacteriophora, Steinernema carpocapase, and Steinernema feltiae. Journal of Nematology, 57(1). https://doi.org/10.2478/jofnem-2025-0020

37. Jaffuel, G., Imperiali, N., Shelby, K., Campos-Herrera, R., Geisert, R., Maurhofer, M., ... & Hibbard, B. E. (2019). Protecting maize from rootworm damage with the combined application of arbuscular mycorrhizal fungi, Pseudomonas bacteria and entomopathogenic nematodes. Scientific reports, 9(1), 3127. https://doi.org/10.1038/s41598-019-39753-7

38. Bütüner, A. K., İlktan, M., & Susurluk, A. (2023). Effects of storage temperature on viability and virulence of entomopathogenic nematodes Heterorhabditis bacteriophora Poinar, 1976 (Rhabditida: Heterorhabditidae), Steinernema carpocapsae Weiser, 1955 and Steinernema feltiae Filipjev, 1934 (Rhabditida: Steinernematidae). Turkish Journal of Entomology, 47(3), 247-257. https://doi.org/10.16970/entoted.1279074

39. Ramakuwela, T., Hatting, J., Laing, M. D., Hazir, S., & Thiebaut, N. (2015). Effect of storage temperature and duration on survival and infectivity of Steinernema innovationi (Rhabditida: Steinernematidae). Journal of Nematology, 47(4), 332.

40. Darwish, I. A. A., Martins, D. P., Ryan, D., & Kakouli-Duarte, T. (2025). State of the art on the interaction of entomopathogenic nematodes and plant growth-promoting rhizobacteria to innovate a sustainable plant health product. Crops, 5(4), 52. https://doi.org/10.3390/crops5040052

41. Fadiji, A. E., Xiong, C., Egidi, E., & Singh, B. K. (2024). Formulation challenges associated with microbial biofertilizers in sustainable agriculture and paths forward. Journal of Sustainable Agriculture and Environment, 3(3), e70006. https://doi.org/10.1002/sae2.70006

42. Li, H., Zhang, K., Zhang, T., & Qian, X. (2026). Effects of Temperature-Moisture Interactions on Storage Survival and Virulence in Two Entomopathogenic Nematode Species. Insects, 17(7), 723. https://doi.org/10.3390/insects17070723

43. Connick Jr, W. J., Nickle, W. R., & Vinyard, B. T. (1993). " Pesta": New Granular Formulations for Steinernema carpocapsae. Journal of Nematology, 25(2), 198.

44. Sharghi, H., Kary, N. E., & Mohammadi, D. (2025). Enhancing the shelf life of entomopathogenic nematodes formulation: The impact of super absorbent polymer and infective juveniles concentration. Crop Protection, 190, 107091.
https://doi.org/10.1016/j.cropro.2024.107091

45. Zulu, S., Ramakuwela, T., Baimey, H., Laing, M., Shapiro-Ilan, D., & Cochrane, N. (2025). Storage capacity of entomopathogenic nematodes in Barricade® gel and potassium polyacrylate hydrogel. Journal of nematology, 57(1).
https://doi.org/10.2478/jofnem-2025-0014

46. Guleria, A., Thakur, H., Kumari, S., & Thakur, N. (2026). Integrating entomopathogenic nematodes and nanotechnology for next-generation insect pest management. Journal of Nanoparticle Research, 28(9), 234. https://doi.org/10.1007/s11051-026-06760-5

47. Thakur, N., Kaur, S., & Jhamta, S. (2025). Next-generation entomopathogenic nematode formulations: advancing sustainable development. Current Microbiology, 82(12), 592. https://doi.org/10.1007/s00284-025-04566-7

48. Kotliarevski, L., Cohen, R., Ramakrishnan, J., Wu, S., Mani, K. A., Amar-Feldbaum, R., ... & Mechrez, G. (2022). Individual coating of entomopathogenic nematodes with titania (TiO2) nanoparticles based on oil-in-water pickering emulsion: a new formulation for biopesticides. Journal of Agricultural and Food Chemistry, 70(42), 13518-13527.
https://doi.org/10.1021/acs.jafc.2c04424

49. Wu, S., Mechrez, G., Ment, D., Toews, M. D., Mani, K. A., Feldbaum, R. A., & Shapiro-Ilan, D. I. (2023). Tolerance of Steinernema carpocapsae infective juveniles in novel nanoparticle formulations to ultraviolet radiation. Journal of Invertebrate Pathology, 196, 107851. https://doi.org/10.1016/j.jip.2022.107851

50. Ramakrishnan, J., Salame, L., Mani, K. A., Feldbaum, R., Karavani, E., Mechrez, G., ... & Ment, D. (2023). Increasing the survival and efficacy of entomopathogenic nematodes on exposed surfaces by Pickering emulsion formulations offers new venue for foliar pest management. Journal of Invertebrate Pathology, 199, 107938. https://doi.org/10.1016/j.jip.2023.107938

51. Ansari, M. A., Hussain, M. A., & Moens, M. (2009). Formulation and application of entomopathogenic nematode-infected cadavers for control of Hoplia philanthus in turfgrass. Pest Management Science: formerly Pesticide Science, 65(4), 367-374.
https://doi.org/10.1002/ps.1699

52. Dolinski, C., Shapiro-Ilan, D., & Lewis, E. E. (2015). Insect cadaver applications: pros and cons. In Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection (pp. 207-229). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-18266-7_8

53. Sandhi, R. K., Shapiro-Ilan, D., Ivie, M., & Reddy, G. V. (2021). Biocontrol of wireworms (Coleoptera: Elateridae) using entomopathogenic nematodes: The impact of infected host cadaver application and soil characteristics. Environmental Entomology, 50(4), 868-877. https://doi.org/10.1093/ee/nvab042

54. Cassells, M. D., Treanor, N., Muñoz-Adalia, E. J., & Griffin, C. T. (2024). Damage to the host cadaver, simulating the effects of scavenging, differentially affects fitness of entomopathogenic nematode species. Journal of Invertebrate Pathology, 207, 108217. https://doi.org/10.1016/j.jip.2024.108217

55. Erdoğan, H., Ünal, H., Susurluk, İ. A., & Lewis, E. E. (2023). Precision application of the entomopathogenic nematode Heterorhabditis bacteriophora as a biological control agent through the Nemabot. Crop Protection, 174, 106429.
https://doi.org/10.1016/j.cropro.2023.106429

56. Ulu, T. C., & Erdoğan, H. (2023). Field application of encapsulated entomopathogenic nematodes using a precision planter. Biological Control, 182, 105240. https://doi.org/10.1016/j.biocontrol.2023.105240

57. Lee-Park, I. B., Lee, J. C., Hodson, A. K., Ganjisaffar, F., Skabeikis, D. D., & Zalom, F. G. (2026). Using drip irrigation to distribute entomopathogenic nematodes for the control of spotted wing drosophila. Biological Control, 212, 105951. https://doi.org/10.1016/j.biocontrol.2025.105951

58. Waweru, B. W., Kajuga, J. N., Hategekimana, A., Ndereyimana, A., Kankundiye, L., Umulisa, C., ... & Toepfer, S. (2025). Formulation of entomopathogenic nematodes for above-ground use against tomato leaf Miner, Phthorimaea absoluta. Insects, 16(2), 189. https://doi.org/10.3390/insects16020189

59. Fallet, P., Bazagwira, D., Guenat, J. M., Bustos-Segura, C., Karangwa, P., Mukundwa, I. P., ... & Turlings, T. C. (2022). Laboratory and field trials reveal the potential of a gel formulation of entomopathogenic nematodes for the biological control of fall armyworm caterpillars (Spodoptera frugiperda). Biological control, 176, 105086. https://doi.org/10.1016/j.biocontrol.2022.105086