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Entomo-Pathogenic Nematode: An Effective and Eco-Friendly Alternative to Synthetic Pesticides. A Review

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ABSTRACT

Entomopathogenic nematodes (EPNs) belong to the category of nematodes living in the soil, which also parasitize and kill insects. They operate in conjunction with specific bacteria and are commonly used in biocontrol because they are effective against a wide range of insect pests, host specific, environmentally safe and compatible with integrated pest management (IPM) strategies. They are obligate parasites of insects that belong to the families Steinernematidae and Heterorhabditidae. EPNs are commonly used in biological pest control due to their broad host range. Entomopathogenic nematodes are effective and eco-friendly alternative to synthetic insecticides. Despite difficulties in mass production, formulation, and field conditions, their incorporation into pest control strategies bears significant promise. Advancements in biotechnology and formulation science can easily promote their applicability in sustainable agriculture. Recent research focuses on improving their field efficiency, genetic diversity, and commercialization. In some countries, EPNs are commercially produced for biological pest control in agriculture, horticulture, and forestry, however, efficient mass production and formulation are critical for their stability, storage, and field efficacy. EPNs are increasingly used in Africa for managing key agricultural pests due to their effectiveness against banana weevils, fall armyworm, sweet potato weevils, and white grubs, but the key challenges include, temperature sensitivity and farmer adoption, however, ongoing research and local production are improving accessibility. Recent studies in some African countries indicated appreciable success achieved with EPN use in Kenya (maize), Uganda (banana), Nigeria (sweet potato), and South Africa (maize grubs) and the key factors for the success include, proper application timing, farmer training, and local EPN adaptation among others.

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INTRODUCTION

Entomopathogenic nematodes (EPNs) are regarded as a group of soil-harbouring nematodes which parasitize and kill insects. They form symbiotic association with particular bacteria and are commonly used in biological control because of their effectiveness against a wide range of insect pests, safety of the environment, and host specificity (Kaya & Gaugler, 1993). Entomopathogenic nematodes are effective and eco-friendly alternative to synthetic insecticides. Despite difficulties in mass production, formulation, and field conditions, their incorporation into pest control strategies bears significant promise. Advancements in biotechnology and formulation science can easily promote their applicability in sustainable agriculture (Grewal et al., 2005). Entomopathogenic nematodes (EPNs) are soil-dwelling, obligate parasites of insects that belong to the families Steinernematidae and Heterorhabditidae (Poinar, 2011). They form symbiotic associations with bacteria (*Xenorhabdus spp*. for *Steinernema* and *Photorhabdus* spp.

for *Heterorhabditis*) to kill insect hosts rapidly (Forst, 2002). EPNs are widely used in biological pest control because of their broad host range, environmental safety, and compatibility with integrated pest management (IPM) strategies (Lacey & Georgis, 2012). Recent research focuses on improving their field efficiency, genetic diversity, and commercialization (Ansari et al., 2010). In some countries, EPNs are commercially produced for biological pest control in agriculture, horticulture, and forestry, however, efficient mass production and formulation are critical for their stability, storage, and field efficacy (Ehlers and Shapiro-Ilan, 2023). Entomopathogenic nematodes (EPNs) are increasingly used in Africa for managing key agricultural pests due to their effectiveness, environmental safety, and compatibility with integrated pest management (IPM) (Kagimu and Malan, 2024). EPNs are effective against banana weevils, fall armyworm, sweet potato weevils, and white grubs in Africa, but the key challenges include, temperature sensitivity and farmer adoption, however, ongoing research and local production are improving accessibility (Abate et al., 2023). Recent studies in some African countries indicated appreciable success achieved with EPN use in Kenya (maize), Uganda (banana), Nigeria (sweet potato), and South Africa (maize grubs) and the key factors for the success include, proper application timing, farmer training, and local EPN adaptation among others (Kagimu and Malan, 2024).

Taxonomy and Classification: EPNs primarily belong to two families namely, Steinernematidae and Heterorhabditidae under the two notable genera, *Steinernema* (e.g., *S. carpocapsae*, *S. feltiae*, *S. glaseri*) and *Heterorhabditis* (e.g., *H. bacteriophora*, *H. indica*, *H. megidis*) and both groups are obligate parasites which carry symbiotic bacteria, *Xenorhabdus* spp and *Photorhabdus* spp. Respectively, (Stock & Goodrich-Blair, 2012).

Few species belonging to the two nematode families suitable for biocontrol:

Steinernematidae Family; Key Genera and Spacies	Heterorhabditidae Family; Key Genera & Species
Steinernema carpocapsae	*Heterorhabditis bacteriophora*
Steinernema feltiae	*Heterorhabditis indica*
Steinernema glaseri	*Heterorhabditis megidis*
Steinernema riobrave	*Heterorhabditis zealandica*
Steinernema scapterisci	*Heterorhabditis amazonensis*
Steinernema kraussei	*Heterorhabditis baujardi*
Steinernema monticolum	*Heterorhabditis floridensis*
Steinernema affine	
Steinernema bicornutum	
Steinernema ceratophorum	

Source: Kaya & Gaugler (2022) modified

Morphology and Life Cycle - The infective juvenile (IJ) stage is the only free-living, non-feeding, and environmentally resistant stage that seeks hosts and they resemble other nematodes but are equipped with a specialized mouth for penetration and symbiont delivery (Koppenhöfer et al., 2020). The infective juveniles penetrate the insect host through natural openings or directly through the cuticle (*Heterorhabditis* spp.), they release symbiotic bacteria into the insect's hemocoel and the bacteria multiply, kill the host within 24–72 hours, and produce antibiotics to suppress competing microbes, while the nematodes feed on the bacterial soup, reproduce inside the cadaver, and after 1–3 generations, new IJs emerge to search for a new host (Grewal et al., 2005).

Comparative Features of Steinernematidae and Heterorhabditidae

FEATURE	Steinernematidae	Heterorhabditidae
Bacterial Symbiont	Xenorhabdus spp.	Photorhabdus spp.
Host Entry	Natural openings	Direct cuticle penetration
Reproduction	Amphimictic (male & female)	Hermaphroditic (self-fertilizing)
Host Cadavers	Not pigmented	Red (due to bacterial pigment)
Optimal Temperature	20-30° C	20-28° C

Source: Campos-Harrera (2023) modified.

Mechanism of Pathogenicity - The insect death is primarily due to Septicemia caused by rapid multiplication of *Xenorhabdus* or *Photorhabdus* spp., toxins released by bacteria that affect insect immunity and physiology and production of secondary metabolites (e.g., proteases, lipases, antibiotics) that break down host tissues for nematode nutrition (Waterfield et al., 2009). This process also prevents decomposition by other microbes and maintains a conducive environment for nematode reproduction (Forst & Clarke, 2002). EPNs also evade insect immune responses by suppressing melanization and phagocytosis (Eleftherianos et al., 2010).

Host Range and Ecological Distribution

EPNs infect a wide range of insect orders, which includes, Coleoptera (e.g., white grubs, *Diabrotica*), Lepidoptera (e.g., cutworms, armyworms) and Diptera (e.g., fungus gnats, *Bradysia* spp.) (Shapiro-Ilan et al., 2017). EPNs thrive in moist, well-aerated soils but are sensitive to UV radiation and extreme temperatures (Kaya & Koppenhöfer, 1996). Some species (*S. carpocapsae*) prefer ambush strategies, while others (*S. glaseri*) use cruising foraging behaviour (Lewis et al., 2012).

Mass Production and Formulation – In Vivo Production is done by infecting larvae of *Galleria mellonella* (wax moth) or other insects**,** which is considered cost-effective for small-scale operations and lab maintenance, while In-Vitro production**,** which involves liquid fermentation and solid culture methods is more suited for commercial-scale production and requires precise control of bacterial phase, oxygen, and pH (Shapiro-Ilan et al., 2001). The commonest types of formulation used include Water-dispersible granules, Gels, Clay or alginate matrix and Diatomaceous earth which enhance shelf-life, ease of application, and field persistence (Koppenhöfer et al., 2020). The liquid fermentation method is the one chosen for EPNs mass-production in larger companies.

Factors affecting the efficacy of EPN as biocontrol agent

EPNs require moisture for movement, while the host require coarse textured soil for aeration and movement, and the larger the host size the greater it allows for multiple reproductive cycle of the EPN, however, the optimal temperature range is between 20-30^0 C and the temperature at extremes becomes lethal to both of them. It is equally lethal to expose the IJs to UV radiation and this is why application is usually done during low light intensity but more importantly, proper timing and application method are critical for effective pest control (Grewal et al., 1994).

Applications of EPN in Integrated Pest Management (IPM)

EPNs have been used to control White grubs (*Phyllophaga spp.*, *Holotrichia spp.*), termites, cutworms (*Agrotis spp.*), fungus gnats, weevils, and borers and they can equally be integrated with botanical insecticides, fungal or bacterial biocontrol agents and low-toxicity synthetic pesticides (Lacey & Georgis, 2012). EPN is also effective against soil-dwelling pests (e.g., *Agriotes* wireworms) (Ansari et al., 2010). It is equally used in orchards, vineyards, and turfgrass (Koppenhöfer, 2007). EPN controls invasive pests like *Anoplophora glabripennis* (Asiatic longhorn beetle) (John et al., 2021). This practice is compatible with reduced chemical insecticide use (Laznik & Trdan, 2016). Many nematode species, such as *S. carpocapsae, S. feltiae, S. glaseri, S. kushidai, S. riobrave, S. scapterisci, H. indica, H. bacteriophora* and *H. megidis* can be successfully mass produced in 7 to 8 litres liquid media in bioreactors (Lulamba et al., 2019)

ADVANTAGES AND LIMITATIONS

Advantages- the application of EPN is safe to humans, animals, and plants, it leads to rapid insect mortality, it can be mass-produced and stored and it is also compatible with IPM programs (Shapiro-Ilan, 2012 and Grewal, 2002).

Limitations- EPN is susceptible to desiccation and UV light, its effectiveness is reduced in dry, hot, or heavily compacted soils, it has narrow spectrum unless used in combination with other agents (e.g. adjuvants- Micron Herbaflex) may cost higher than synthetic pesticides and has rare but possible effects on beneficial arthropods (Pekar, 2021).

Remedies- EPNs are UV-Sensitive and require evening/night spraying, most importantly is the formulation of additives such as the use of anti-desiccants (e.g., kaolin clay) to improve survival of the symbionts, as well as the use of maize seeds coated with alginate-encapsulated EPNs for early pest control (Kagimu & Malan, 2024).

Control of Tropical Insect Pests in Africa Using Entomopathogenic Nematodes

a) Major Soil-dwelling Insect Pests Controlled by EPNs in Tropical Africa

Pest	Crop Affected	EPN spp. used	Efficacy
Banana weevil	Banana, Plantain	*H. indica; S. carpocapsae*	70-90% larval mortality
Sweet potato weevil	Sweet potato	*H. bacteriophora; S. feltiae*	Up to 80% Reduction
White grubs	Maize; Sorghum	*H. baujardi; S. riobrave*	60-85% control
Termites	Multiple crops	*H. sonorensis; S. virgalemense*	Moderate (50-70%)

Source: Kagimu & Malan (2024) modified.

b) Foliar and Stem-borer Pests Controlled by EPN in Tropical Africa

Pest	Crop Affected	EPN spp. used	Efficacy
Fall Army worm	maize	*H. zealandica; S. carpocapsae*	50-75% larval killing
Stem Borers	Maize; Sorghum	*S. feltiae*	Limited (soil applied)
Fruit fly larvae	Mango	*S. virgalenmense*	Emerging research

Source: Kagimu & Malan (2024) modified.

African Countries where EPNs were successfully applied against tropical insect pests:

Country	Host	Target crop	EPN Used	Appl. Mthd	Result
Kenya	Fall Armyworm	Maize	*S. carpocapsae*	Soil drench collar base	70-80% L. mortality
Uganda	Banana weevil	Plantain, banana	*H. indica* Ugan. Strain	Soil injection collar base	80% reduction
Nigeria	S. potato weevil L.	Sweet potato	*H. bacteriophora*	Tuber dip + soil drench	78% reduction
S/Africa	White grubs	Maize root	*H. zealandica*	Broadcast at planting	65% reduction
Ghana	Cassava mealybug	Cassava root (experiment)	*S. feltiae*	Soil drench (collar)	50-60% suppression
Ethiopia	Wheat Stem borer	Larva in wheat stem	*S. yirgalemense*	Foliar spray + soil drench	55% larval mortality

Sources: Mwaniki (2022), Abate (2023), Ekeh (2021), Dlamini (2022), Atiri (2023), Tadesse (2021)

Molecular Advances and Future Prospects

EPN has undergone genetic enhancement where by selection for heat-tolerant strains is possible (Yasur et al., 2020). Nanotechnology has made encapsulation possible to improve UV resistance (Khashan et al., 2021). Genomic studies are embarked upon to identify virulence factors and improve EPN resistance (Bait et al., 2013). Genomic studies to understand host-pathogen interactions and improve EPN resilience and the efforts to produce transgenic symbionts that secrete additional toxins are ongoing but face regulatory hurdles (Chaston et al., 2011). Future prospect include development of region-specific EPN strains for local pests, enhancing shelf-life and field persistence through better formulations, use in urban pest control, turf, greenhouse, and forestry, combination with drones or precision applicators for large-scale deployment and further exploration of EPN-bacteria-insect tri-trophic interactions using omics technologies (Chaston et al., 2011). Modern application methods have been developed to reduce desiccation of foliar-applied EPNs, ranging from the post-application spraying of a gel that was originally used in firefighting, to the envelopment of treated areas with moistened diapers. Simple management practices, such as altering the time of application to either late in the evening or in the early morning, can play an important role in attaining nematode efficacy, as nematodes need only a few hours of optimum conditions to be able to infect the host (Platt et al., 2019).

CONCLUSION

Entomopathogenic Nematodes are very effective biocontrol agents because of their symbiotic bacteria, rapid host-killing capability, and broad host range. Recent research focuses on improving their field efficacy, genetic diversity, and commercialization. EPNs are produced via in-vivo (insect-based) and in-vitro (solid/liquid culture) methods. Formulations comprises of granules, gels, and liquid suspensions to enhance stability and application efficiency. Recent advances focus on bioreactor scaling, UV protection, and cryopreservation. EPNs are effective against banana weevils, fall armyworm, sweet potato weevils, and white grubs in Africa. Key challenges include temperature sensitivity and farmer adoption, but ongoing research and local production are improving accessibility. If farmers are encouraged it will be a sustainable means of crop protection among farmers.

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