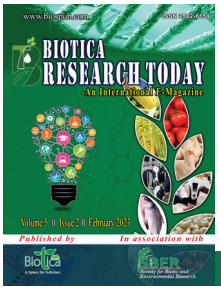
Bio. Res. Today 2023, 5(2):199-203



Biotica Research Today

e-ISSN: 2582-6654

February, 2023

Article ID: RT1256 **Popular Article**

Entomopathogenic Nematodes: Mode of Action and Application Methods

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Conflict of interests: The author has declared that no conflict of interest exists.

How to cite this article?

Prasad and Singh, 2023. Entomopathogenic Nematodes: Mode of Action and Application Methods. *Biotica Research Today* 5(2), 199-203.

Abstract

Entomopathogenic nematodes are obligate parasites of insects and are used as biological control agents of economically important insect pests. They occur naturally in soil environments and locate their host in response to carbon dioxide, vibration and other chemical cues. Synthetic chemical pesticides are routinely employed to manage insect pests, but they have a negative influence on our environment and the systems of non-targeted beneficial organisms, including humans. Entomopathogenic nematodesas bio-pesticides are a safe, natural, and cost-effective alternative to replace the hazardous chemical pesticides. Due to a lack of innovative advancements in research and policies in India, there are currently few entomopathogenic formulations commercially available, which are insufficient to fulfil farmer demand. Comparatively synthetic chemical pesticides, entomopathogen production and usage are very minimal. This article covers different entomopathogenic nematodes, their mode of action and application methods for management of economically important insect-pests in an eco-friendly manner.

Keywords: Biological control, Entomopathogenic nematodes, EPNs, Mode of action

Introduction

Entomopathogenic nematodes (EPNs) are a group of nematodes (thread worms), that kill to insects. The term entomopathogenic has a Greek origin, with entomon, meaning insect, and pathogenic, which means causing disease. EPNs live parasitically inside the infected insect host, and so they are termed as *endoparasitic*. They infect many different types of insects living in the soil like the larval forms of moths, butterflies, flies and beetles as well as adult forms of beetles, grasshoppers and crickets. They have been found in all over the world and a range of ecologically diverse habitats. EPNs were first identified in the 1920s, and their commercialization began in the 1980s. EPNs are beneficial nematodes attack soil-borne insect pests while being non-harmful to humans, animals, plants, or earthworms, and can thus be utilised as biological control organisms. Nematodes are worm-like unsegment invertebrates that are found all over the world. Entomopathogenic nematodes belong to order Rhabditida and families Heterorhabditidae and Steinernematidae present naturally in soil habitat and find their host *via* physical and chemical signal responses (Shapiro-Ilan *et al.*, 2012). Both of these families have species, which have been successfully used as bio-insecticides in pest management programs. DD-136 strain of *Steinernema carpocapsae* in India was first demonstrated for the management of lepidopteran insect pests of apple, rice and sugarcane. Farmers utilise species-specific EPN-based products such as Biovector, Sanoplant, Helix, Magnet, and Entonem, which are available in developed countries. Only two formulations, "Green Commandos" and "Soil Commandos," were developed in India utilising foreign EPN species; however, these nematodes were ineffective against insects, most likely due to their poor adaption to Indian climatic conditions. Currently, Multiplex Biotech Pvt. Ltd.'s latest formulations are advertised as Soldier (contains *Heterorhabditis indica*) and Bouncer (contains *Steinernema carpocapsae*), although these formulations are not widely used by farmers. Currently, *Steinernema carpocapsae,*

Article History

RECEIVED on 17th February 2023 RECEIVED in revised form 25th February 2023 ACCEPTED in final form 26th February 2023

Steinernema feltiae, Steinernema kraussei, Steinernema glaseri, Steinernema riobrave, Heterorhabditis bacteriophora and *Heterorhabditis megidis* are the most commonly used and successfully applied nematodes due to the fact that they can easily be produced in liquid culture (Abate *et al.*, 2017).

The indiscriminate use of chemical pesticides for insect control in various agro-ecosystems has produced several environmental problems, including ground water contamination, residue in food, resistance development, soil pollution, air pollution, secondary pest breakout, pest resurgence, and so on. EPNs have become increasingly popular as a pesticide option because of their environmentally favourable features and bio-control agent like behaviour. Applications of most of the EPNs are suitable with sprayers that are pressurised, mist, electrostatic, fan, or aerial. Watering cans, hose-end sprayers, and pump sprayers are all excellent applicators. During crop growing seasons, nematodes can also be sprayed to agricultural fields through irrigation systems. Several EPNs (Table 1, 2 and 3) are commercially used for management of important insect-pests (Prasad *et al.*, 2007; Shapiro-Ilan and Gaugler, 2010; Gozel and Gozel, 2016).

Mode of Action

Generally, third stage of EPNs, known as the infective juvenile (IJ) of both nematodes *i.e., Steinernema* and *Heterorhabditis*, is considered infective and pathogenic. *Xenorhabdus* and *Photorhabdus* are entomopathogenic bacteria (EPB) vectored by *Steinernema* and *Heterorhabditis* genera of EPNs, respectively. The sole free-living stage of EPNs is infective juveniles, while other developing stages can only be seen inside infected insect hosts. Infective juveniles enter the hemocoel of the host insect by natural apertures like as spiracles, mouth, and anus, or in certain species through inter-segmental membranes of the cuticle. In addition to natural holes, *Heterorhabditis* species can enter the insect host by abrading the skin. The infective juveniles enter the epidermis and release symbiotic bacterial cells from their alimentary canal (intestine) into the hemocoel. After piercing the epidermis, the IJs discharge symbiotic bacteria cells from their alimentary canal into the hemocoel. The bacteria proliferate in the insect haemolymph, release poisons and specific immune depressors, and the insect's immune system is suppressed, resulting in death within 24-48 hours. *Photorhabdus* and *Xenorhabdus*, two well-known bacterial symbionts of EPNs, are not only toxic to entomic fauna, but they also inhibit opportunistic bacteria and fungi.

Table 1: EPN formulations developed in India

Sl. No.	EPN Species	Formulation/ Products name
1	*Steinernema bicornutum*	Bait as alginate capsule
2	*Steinernema abbasi*	Hydrogel
3	*Heterorhabditis indica*	Spray formulations talk-based, Soldier,
4	*Steinernema abbasi*	Talk based
5	*Steinernema carpocapsae*	Spray formulations talk-based, Bouncer, Alginate capsule, Wheat bran pellets, Pearl (Sodium alginate- based)
6	*Steinernema riobrave*	Spray adjuvants

Table 2: EPN formulations developed by other countries

Sl. No.	EPN Species	Formulation/ Products name	Country
1	*Steinernema feltiae*	Magnet	USA
		Entoname	Netherland
		Nemasys, Stealth	UK
		Exhibit	Switzerland
		Agrifutur	Italy
		SDS Biotech	Japan
3	*Steinernema riobrave*	Vector MG, Biovector	USA
		Biovector	Columbia
4	*Heterorhabditis bacteriophora*	E-Nema Gmbh	Germany
		Otinem	USA, Switzerland
		Soil commondos	India
		Nemopak HB	Italy
5	*Steinernema scapterisci*	Proactant Ss	USA
6	*Phasmarhabditis hermaphrodita*	Nemaslug	UK
7	*Heterorhabditis indica*	Soldier	India

Table 2: Continue...

Sl. No.	EPN Species	Formulation/ Products name	Country
8	*Heteroshabditis megidis*	Nemasya	UK
		Larvanem	Netherland
		NovoNem	Germany
9	*Steinernema carpocapsae*	ORTHO Biosafe, Biovector, X-GNAT, Ecomask, Heteromask	USA
		Biovector	Columbia
		Green commonda, Bouncer	India
		Helix	Canada
		CAPSANEM	Netherland
		Mioplant	Austria
		Sanoplant	Switzerland
		Nemastar	Italy

Table 3: EPNs commercially used for biological control of some insect-pests

Name of pests	Scientific name	Targeted crops	Effective nematode species
Corn rootworm	*Diabrotica* spp.	Vegetables	*Steinernema carpocapsae, Heterorhabditis bacteriophora*
Artichoke plume moth	*Platyptilia carduidactyla*	Artichokes	*Steinernema carpocapsae*
White grub	*Holotrichia consanguinea*	Potato, Sugarcane, Groundnut	*Steinernema carpocapsae, S. glaseri*
Rice moth	*Corcyra cephalonica*	Rice	*Steinernema carpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
Banana root borer	*Cosmopolites sordidus*	Bananas	*Steinernema carpocapsae, S. feltiae, S. glaseri*
Corn earworm	*Helicoverpa zea*	Vegetables	*Steinernema carpocapsae, S. feltiae, S. riobrave*
Greater wax moth	*Galleria mellonella*	Worker honey bee	*Steinernema carpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
Blue butterfly	*Lampides boeticus*	Black and Green gram	*Steinernema carpocapsae, S. seemae, S. masoodi*
Diamond black moth	*Plutella xylostella*	Cabbage	*Steinernema carpocapsae*
Citrus root weevil	*Pachnaeus* spp.	Citrus, Ornamentals	*Heterorhabditis bacteriophora, S. riobrave*
Gram pod borer	*Helicoverpa armigera*	Chickpea, Pigeonpea, Tomato, Field pea	*Steinernema carpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
Armyworm	*Spodoptera frugiperda*	Vegetables	*Steinernema carpocapsae, S. feltiae, S. riobrave*
Black cutworm	*Agrotis ipsilon*	Turf, vegetables	*Steinernema carpocapsae*
Shore fly	*Scatella* spp.	Ornamentals	*Steinernema carpocapsae, S. feltiae*
Black vine weevil	*Otiorhynchus sulcatus*	Berries, Ornamentals	*Heterorhabditis bacteriophora, H. downesi, H. morelata, H. megidis, Steinernema carpocapsae, S. glaseri*
Navel orange worm	*Amyeloistran sitella*	Nut and fruit trees	*Steinernema carpocapsae*
Mole cricket	*Scapteriscus* spp.	Turf	*Steinernema carpocapsae, S. riobrave*
Small hive beetle	*Aethina tumida*	Bee hives	*Heterorhabditis indica, S. riobrave*
Root weevil	*Otiorhynchus ovatus*	Berries strawberry	*Heterorhabditis marelata*
Plum curculio	*Conotrachelus nenuphar*	Fruit trees	*S. riobrave*

Table 3: Continue...

Name of pests	Scientific name	Targeted crops	Effective nematode species
Sweet potato weevil	*Cylas formicarius*	Sweet potato	*Steinernema carpocapsae, Heterorhabditis bacteriophora, S. feltiae*
Iris borer	*Macronoctua onusta*	Iris	*Heterorhabditis bacteriophora, Steinernema carpocapsae*
Fungus gnat	*Dip: Sciaridae*	Mushrooms	*Heterorhabditis bacteriophora, S. feltiae*
Banana moth	*Opogona sacchari*	Ornamentals, Banana	*Steinernema carpocapsae, Heterorhabditis bacteriophora*
Leaf miner	*Liriomyza* spp.	Vegetables, Ornamentals	*Steinernema carpocapsae, S. feltiae*
Codling moth	*Cydia pomonella*	Pome fruit	*Steinernema carpocapsae, S. feltiae*
Cranberry girdler	*Chrysoteuchia topiaria*	Cranberries	*Steinernema carpocapsae*
Billbug	*Sphenophorus* spp.	Turf	*Heterorhabditis bacteriophora, Steinernema carpocapsae*
Grape root borer	*Vitacea polistiformis*	Grapes	*Heterorhabditis bacteriophora, H. zealandica*
Tobacco caterpillar	*Spodoptera litura*	Tobacco	*Steinernema carpocapsae, S. feltiae, S. abbasi, Heterorhabditis indica*

Application Methods

EPN can be delivered along with pesticides or fertilizers or they can also be used with the irrigation system. Small pressured sprayers, mist blowers, and fan sprayers are utilized for application of EPNs. The pressure in the spray tank should not be too high, otherwise the EPN will shred into small pieces. EPN can sustain pressures of up to 300 pounds inch^{-2} and is compatible with all standard nozzle types, with openings as little as 50 microns in diameter.

1. Foliar Application

Spraying in standing agricultural plants is one of the most prevalent techniques of EPN application. The kind of nozzle utilised, droplet size, and spray distribution mechanism all influence EPN deposition on plant surfaces. Adjuvant addition promotes EPN deposition on foliage. To overcome the extremities of high temperature and UV radiation adjuvant are required. The addition of fluorescent brighteners such as Tinopal, Ujala, Ranipal @ 0.01% or Glycerine @ 1% and others can provide effective protection against these environmental influences (Prasad *et al.*, 2007). Spray of 1000 IJs plant^{-1} (equivalent to 125 million IJs ha^{-1}) or @ 1×10^{6} IJs ml^{-1} or 3×10^{9} IJs ha^{-1} is done as a prophylactic control measure. Inject 20 ml of active infective juveniles (IJs) of EPN @ 1,000 IJs ml^{-1} in to the holes as curative measure for management of stem borer/ pseudostem weevil/ banana stem weevil (*Odoiporous longicollis*). Generally, frass materials or jelly like exudations are observed on the holes of pseudostem in such type of infestation.

2. Soil Application

Comparatively, sandy loan soil is preferable than clay soil for nematode migration and survival. When using EPN, the temperature of the soil is also taken into consideration. If the soil temperature is higher than 28 °C, a mild pre-irrigation is normally recommended to bring the temperature down. A light irrigation is also often suggested after adding EPN to soil so that the nematode may move deeper into the soil and act efficiently (Prasad *et al.*, 2007). Also of paramount importance, to be effective, EPNs usually must be applied to soil at minimum rates of 2.5×10^{9} IJs ha^{-1} (= 25 cm^{-2}) or higher. Before application of EPN, irrigate the field before 2-3 days to maintain the soil moisture or if required moisture level is already present in the field then EPN can be applied. While preparing the field, 2-5 kg acre^{-1} EPN culture (powder formulation) is added in 20-50 kg well decomposed farm yard manure (FYM) or vermin-compost/ coco-pit/ sand. 5-25 g EPN is required for plantation and fruit crops plant^{-1}.

3. Soil Drenching

In drenching, EPNs are mostly applied in root zone area of the plants at the rate of 10 g mixture litre^{-1} of clean water. Nozzle of Knapsack pump is removed during the application of EPN formulation in the soil.

Conclusion

EPNs are obligate parasites of insects and used as biological control agents of economically important insect pests. They are commercially used as safe alternatives to chemical insecticides. Opportunities that enabled their development and implementation for the control of insect pests should be better exploited. Infective juveniles of EPNs actively seek out their hosts and enter through natural openings such as the mouth, spiracles, anus or through the inter-segmental membrane and kill the insects within 24-48 hours. Its infective juveniles are usually applied using various spray equipment and standard irrigation systems. Enhanced efficacy in EPN applications can be facilitated through improved delivery mechanisms or optimization of spray equipment. EPNs are compatible with most of the agrochemicals including fungicides, insecticides, herbicides, and acaricides, and may therefore be blended in the tank. Mancozeb and neem are safe to most of the nematode populations.

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