Entomopathogenic Nematode: A Potential Bio-Control Agent for Sustainable Crop Production

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Abstract

Agriculture is vital to India's economy. Insect infestation significantly reduces agricultural crop productivity. To satisfy the demands of India's and other emerging countries' growing populations, the Indian agricultural system requires sustainable agricultural production for future generations. This article discusses the current state of Entomopathogens in the agriculture sector. 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. Entomopathogens as bio-pesticides are a safe, natural, and cost-effective alternative to replace those dangerous 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. Particularly compared to synthetic chemical pesticides, entomopathogen production and usage are minimal. This article recommends improving technologically focused research and raising entomopathogenic bio-pesticide formulations to increase agricultural productivity in an environmentally responsible manner.

Keywords: Entomopathogenic nematodes, *Heterorhabditisindica*, *Steinernemacarpocapsae*, mode of action, biological control

Introduction

The word "entomopathogenic nematodes" (EPNs) is derived from the Greek vocables, ‘Εντομος’ (entomos, ‘insect’) ‘πάθη’ (pathê, ‘disease’) and ‘γ΄ενος’ (guenos, ‘producing’),

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means a group of nematodes which have the ability to cause disease in insects by suppressing the immune system of insects. Entomopathogenic nematodes (EPNs) were first identified in the 1920s, and their commercialization began in the 1980s. Entomopathogenic nematodes 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. They have a wide variety of habitats, including soil, water (fresh and salt), plants, and animals. Entomopathogenic nematodes (EPNs) are the microscopic worms that have the ability to kill insects. Entomopathogenic nematodes belong to order Rhabditida and families Heterorhabditidae and Steinernematidae present naturally in soil habitat and find their host via signal responses (chemical and physical) (Shapiro-Ilan*et al.*2012). Both of these families have species, which have been successfully used as bio-insecticides in pest management programs. Rao and Manjunath (1966) first demonstrated DD-136 strain of *Steinernemacarpocapsae* in India 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 *Heterorhabditisindica*) and Bouncer (contains *Steinernemacarpocapsae*), although these formulations are not widely used by farmers. Currently, *Steinernemacarpocapsae, Steinernemafeltiae, Steinernemakraussei, Steinernemaglaseri, Steinernemariobrave, Heterorhabditisbacteriophora* and *Heterorhabditismegidis* 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. Because of their environmentally favourable features, biological control agents like as entomopathogenic nematodes have

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become increasingly popular as a pesticide option. In most circumstances, no specialised application equipment is required. Most nematode species 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. Commercial use of EPNs reported by Prasad *et al.* (2007), Shapiro-Ilanand Gaugler (2010) and Gozel and Gozel (2016) against some insect-pests and other targets are given in Table 1, 2 and 3.

Mode of action

Only the 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 developmental stages can only be seen inside infected insect hosts. Infective juveniles enter the homocoel 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.Apart from natural holes, *Heterorhabditis*species can also enter the insect host by abrading the skin. After piercing the epidermis, the IJs discharge symbiotic bacteria cells from their alimentary canal into the hemocoel. The bacteria proliferate in the insect hemolymph, 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 from using the nutrient-rich carcass, effectively trapping.

Table 1: EPN formulations developed and tested in India

SN	EPN Species	Formulation/Tested/Products name
1.	*Heterorhabditisindica*	Spray formulations talk-based, Soldier,
2.	*Steinernemacarpocapsae*	Spray formulations talk-based, Bouncer, Alginate capsule, Wheat bran pellets, Pearl (Sodium alginate- based),Vermiculite-based

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3.	*Steinernemaabbasi*	Talk based
4.	*Steinernemabicornutum*	Bait as alginate capsule
5.	*Steinernemariobrave*	Spray adjuvants
6.	*Steinernemaabbasi*	Hydrogel

Table 2: EPN formulations developed by different countries

SN	EPN Species	Formulation/Products name	Country
1.	*Heterorhabditisbacteriophora*	E-NemaGmbh	Germany
		Otinem	USA, Switzerland
		Soil commondos	India
		Nemopak HB	Italy
2.	*Steinernemacarpocapsae*	ORTHO Biosafe, Biovector, X-GNAT, Ecomask, Heteromask	USA
		Biovector	Columbia
		Green commonda, Bouncer	India
		Helix	Canada
		CAPSANEM	Netherland
		Mioplant	Austria
		Sanoplant	Switzerland
		Nemastar	Italy
3.	*Steinernemafeltiae*	Magnet	USA
		Entoname	Netherland
		Nemasys, Stealth	U. K.
		Exhibit	Switzerland
		Agrifutur	Italy
4.	*Steinernemakushidai*	SDS Biotech	Japan
5.	*Steinernemariobrave*	Vector MG, Biovector	USA
		Biovector	Columbia
6.	*Heterorhabditisindica*	Soldier	India

7.	*Heteroshabditismegidis*	Nemasya	UK
		Larvanem	Netherland
		NovoNem	Germany
8.	*Steinernemascapterisci*	ProactantSs	USA
9.	*Phasmarhabditishermaphrodita*	Nemaslug	UK

Table 3:Globally commercial use of EPN against some insect-pests as bio-control agents

Pests/Common Name	Scientific name	Crops/Targeted	Effective nematode species
Artichoke plume moth	*Platyptiliacarduidactyla*	Artichokes	*Steinernemacarpocapsae*
Black cutworm	*Agrotisipsilon*	Turf, vegetables	*Steinernemacarpocapsae*
Rice moth	*Corcyra cephalonica*	Rice	*Steinernemacarpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
Gram pod borer	*Helicoverpaarmigera*	Chickpea, Pigeon pea, Tomato, Fieldpea	*Steinernemacarpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
Greater wax moth	*Galleria mellonella*	Worker honey bee	*Steinernemacarpocapsae, S. glaseri, S. thermophilum, S. seemae, S. masoodi*
White grub	*Holotrichiaconsanguinea*	Potato, Sugarcane, Groundnut	*Steinernemacarpocapsae, S. glaseri*
Blue butterfly	*Lampidesboeticus*	Black and Green gram	*Steinernemacarpocapsae, S. seemae, S. masoodi*
Diamond black moth	*Plutellaxylostella*	Cabbage	*Steinernemacarpocapsae*
Tobacco	*Spodopteralitura*	Tobacco	*Steinernemacarpocapsae, S.*

caterpillar			*feltiae, S. abbasi, Heterorhabditisindica*
Armyworm	*Spodopterafrugiperda*	Vegetables	*Steinernemacarpocapsae, S. feltiae, S. riobrave*
Banana root borer	*Cosmopolites sordidus*	Bananas	*Steinernemacarpocapsae, S. feltiae, S. glaseri*
Banana moth	*Opogonasacchari*	Ornamentals, Banana	*Steinernemacarpocapsae, Heterorhabditisbacteriophora*
Plum curculio	*Conotrachelusnenuphar*	Fruit trees	*S. riobrave*
Sweet potato weevil	*Cylasformicarius*	Sweet potato	*Steinernemacarpocapsae, Heterorhabditisbacteriophora, S. feltiae*
Root weevil	*Otiorhynchusovatus*	Berries strawberry	*Heterorhabditismarelata*
Leaf miner	*Liriomyza*spp. (dip: Agromyzidae)	Vegetables, Ornamentals	*Steinernemacarpocapsae, S. feltiae*
Mole cricket	*Scapteriscus* spp.	Turf	*Steinernemacarpocapsae, S. riobrave*
Grape root borer	*Vitaceapolistiformis*	Grapes	*Heterorhabditisbacteriophora, H. zealandica*
Corn rootworm	*Diabrotica spp.*	Vegetables	*Steinernemacarpocapsae, Heterorhabditisbacteriophora*
Corn earworm	*Helicoverpazea*	Vegetables	*Steinernemacarpocapsae, S. feltiae,S. riobrave*
Citrus root weevil	*Pachnaeus*spp.	Citrus, Ornamentals	*Heterorhabditisbacteriophora,S. riobrave*
Shore fly	*Scatella* spp.	Ornamentals	*Steinernemacarpocapsae, S. feltiae*
Black vine	*Otiorhynchussulcatus*	Berries,	*Heterorhabditisbacteriophora, H.*

weevil		Ornamentals	*downesi, H. morelata, H. megidis, Steinernemacarpocapsae, S. glaseri*
Navel orange worm	*Amyeloistransitella*	Nut and fruit trees	*Steinernemacarpocapsae*
Small hive beetle	*Aethinatumida*	Bee hives	*Heterorhabditisindica,S. riobrave*
Plum curculio	*Conotrachelusnenuphar*	Fruit trees	*S. riobrave*
Iris borer	*Macronoctuaonusta*	Iris	*Heterorhabditisbacteriophora,Steinernemacarpocapsae*
Fungus gnat	Dip: Sciaridae	Mushrooms	*Heterorhabditisbacteriophora,S. feltiae*
Codling moth	*Cydiapomonella*	Pome fruit	*Steinernemacarpocapsae, S. feltiae*
Cranberry girdler	*Chrysoteuchiatopiaria*	Cranberries	*Steinernemacarpocapsae*
Billbug	*Sphenophorus* spp. (Col: Curculionidae)	Turf	*Heterorhabditisbacteriophora, Steinernemacarpocapsae*
Cat flea	*Ctenocephalidesfelis*	Home yard, turf	*Steinernemacarpocapsae*

Application Technology

The application of EPN in soil and plants requires additional caution in handling so that the negative effects of the environment are minimised and they perform efficiently. EPN effectiveness is reduced if post-application survival is poor. Several methods have been explored to solve this problem, including (i) desiccated cadavers (ii) capsules and (iii) baits.

EPN can be delivered along with pesticides or fertilizers. They can also be used with an irrigation system. Small pressured sprayers, mist blowers, and fan sprayers are utilized in such situations. 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 per square inch and is compatible with all standard nozzle types, with openings as little as 50 microns in diameter.

1. **Soil Application:**In general, 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 28^0C, 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 x 10^9 IJs/ha (=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 EPN culture (powder formulation) is added in 20-50 kg well decomposed farm yard manure (FYM) or vermin-compost/cocopit/sand. 5-25 gram EPN is required for plantation and fruit crops/plant.
2. **Soil Drenching:** Maintain the soil moisture and mix 10 gram/litre of clean water. Mix thoroughly. While drenching, remove the nozzle of Knapsack pump and apply EPN at the root zone area.
3. **Foliar application/Stem borer management:**One of the most prevalent techniques of EPN application is spraying in standing agricultural plants. 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 per plant (equivalent to 125 million IJs/ha) or @ $1x10^6$ IJs/mlor $3x10^9$IJs/haas a prophylactic control measure. For stem borer/Pseudostem weevil/Banana stem weevil (*Odoiporouslongicollis*) management, the holes along with frass material or jelly like exudations are observed on pseudostem, inject 20ml of active infective juveniles (IJs) of EPN @1000IJs/ml in to the holes as curative measure.

Compatibility with Pesticides

EPN are tolerant to most agrochemicals, including fungicides, insecticides, herbicides, and acaricides, and may therefore be blended in the tank. Many hazardous compounds only have a temperature impact because the nematodes recover fast after being exposed to them. Thus, when exposures are short, simultaneous use of nematode with pesticides and fertilizers should be feasible.*S. glaseri* nematode is compatible with carbofuran, quinalphos while *S. carpocapsae* is compatible with dimethoate, endosulfan, mancozeb, malathion and zineb.Mancozeb and neem are safe to all nematode populations except *H. indica*.Neem products like Achook and Neem Gold are compatible with *S. carpocapsae* (Prasad *et al.*, 2007).

Advantages:

1. Highly toxic to important insect pests with little off-target effects.
2. Humans and the environment are quite safe. There is no need for safety equipment, there are no residual effects, there is no groundwater contamination, and pollinators are not harmed.
3. Kills insects within 24-48 hours.
4. They are simple to mass produce and may be applied using normal agrochemical equipment and irrigation systems.

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