

## ENTOMOPATHOGENIC NEMATODES AS BIOINSECTICIDES – A REVIEW

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(Received 14<sup>th</sup> Jan 2021; accepted 18<sup>th</sup> Mar 2021)

**Abstract.** Entomopathogenic nematodes (EPNs) belonging to the *Heterorhabditis* and *Steinernema* genera provide effective bio-control of insect pests. They are extremely lethal to such pests due to mutualistic association with the genera of bacteria *Photorhabdus* and *Xenorhabdus* and are safe for beneficial insects. They are used as biopesticides because they are eco-friendly with no harmful effects on human wellbeing. EPNs are well-suited with the number of agrochemicals and a cost-effective substitute for chemical insecticides. This review assesses the use of EPNs under agroclimatic conditions, the influence of abiotic factors, life cycle, trapping, production, storage, mechanisms, biopesticides and their suitable application. However, a previous study on EPNs and their role as biopesticides have been surveyed for their possible usage as biological control agents.

**Keywords:** *Heterorhabditis*, *Steinernema*, *Photorhabdus*, *Xenorhabdus*, agrochemicals, biological control agent

### Introduction

Nematodes are roundworms, colourless microorganisms, possessing almost all schemes of higher organisms except circulatory or respiratory systems (Sikandar et al., 2019, 2020b). They may be parasitic, predaceous, or free-living and have an abundance of various associations from useful to harmful (Ferris et al., 2012). Their association can be divided into four basic groups; facultative parasitism, obligate parasitism, necromenic and phoretic (Askary et al., 2018). Entomopathogenic nematodes (EPNs) can be obligate or facultative parasites on harmful insects. They have been recorded on all continents except Antarctica (Abate et al., 2017). Entomopathogenic nematodes have a wide host range and can easily find their suitable host. Biological control is a safe way to control pests and pathogens (Sikandar et al., 2020a). They have huge consideration in the field of biological control (Davari and Parker, 2018; Trdan et al., 2020).

Entomopathogenic nematodes are harmless or safe for non-targeted organisms (Dutka et al., 2015) and yet pose no threat to beneficial insects (Akhurst and Smith, 2002). They are eco-friendly and non-toxic to humans and can easily apply with pesticide equipment (Shapiro-Ilan et al., 2006). Steiner in 1923 described the first entomopathogenic nematode (EPNs) (Dillman, 2013).

Twenty-three families of nematodes have been recorded as a parasite of insects but the species of seven families have more potency to control insects (Shapiro-Ilan et al., 2012). Families Heterorhabditiae and Steinernematidae have been more frequently and effectively used in pest biological control (Bal and Grewal, 2017). Usually, species of genera *Steinernema* and *Heterorhabditis* (Rhabditida) are used as biological control agents in the domain of plant protection (Laznik and Trdan, 2014). Species of genus *Oscheius* are entomopathogenic because they are also a parasite of insect pest meanwhile Steinernematidae and Heterorhabditidae received more attention as effective biocontrol agents (Dillman et al., 2012b). The symbiosis of EPNs with bacteria like *Photobacterium* and *Xenorhabdus* can effectively control Coleopteran, Dipteran and Lepidopteran pests (Mohan, 2015).

Entomopathogenic nematodes have been divided into two categories according to their host searching behaviour including cruisers and ambushers. Cruisers such as *Heterorhabditis bacteriophora* and *Steinernema glaseri* are subterranean, more active to find suitable host, while ambushers like *Steinernema carpocapsae* usually wait to attack suitable host in the upper surface of the soil (Mohan, 2015). They can search their host in different ways like vibration, carbon dioxide, or other chemicals (Lortkipanidze et al., 2016). They showed significant potential as natural pest control agents in the soil environment (De Brida et al., 2017). After finding a suitable host and penetrating it, they can kill the host within 1-4 days. Their killing capacity depends upon the host and nematode species.

More than 90% of insects have their life stages in the soil, so are easily exposed to EPNs (Radová, 2010). They can suppress a huge variety of commercially targeted important pests (Lacey and Georgis, 2012). Entomopathogenic nematodes can control a variety of insect species i.e. *Alissonolurn impressicalla*, *Arbela dea*, *Blitoportha pallidipennis* Reitter, *Holotrichia parallela*, *Odoiporus longicollis*, *Otiorynchus sulcatus*, *Pachraetus litus*, *Paranthrene tubaniformis*, *Phylloreta striolul*, etc in China (Mahmoud, 2016). Substantial progress in the application, production and research of entomopathogenic nematodes has been made in the last decade (Lacey et al., 2015).

### **Influence of abiotic and biotic factors**

Entomopathogenic nematodes can survive under a variety of environments but some abiotic factors influence their activity such as infectious juveniles (IJs) can survive in the value of pH of soil between 4-8 but their activities are minimized at a pH value of 10. Moisture and pH also influenced their occurrences (Stuart et al., 2015). During a survey on EPNs in North China, the environmental conditions imposed harmful effects on their virulence, survival, and reproduction (Ma et al., 2010). High salinity also affected the activity of EPNs (Kergunteuil et al., 2016), but it increased the rate of tolerance toward high temperatures (Hussaini, 2017).

They are distributed throughout the world and they can tolerate overwintering conditions, as reported from extreme cold Heilongjiang Province of China (Chunjie et al., 2011). Their recovery depends upon the texture and characterization of soil

(Noosidum et al., 2010). They found usually less in clay soil due to the low oxygen value and matter contents in it. They are more frequently recovered in sandy soil (Banu, 2017). Entomopathogenic nematodes were isolated from sandy soil in South China and Beijing areas (Griffin et al., 2000).

The effects of biotic factors (like species of EPNs, soil fauna, age of targeted insects) and abiotic factors (moisture, temperature, soil type and aeration) have been reported by several researchers (Shapiro-Ilan et al., 2012). Researchers have mainly focused on their potential as inductively applied augmentative biocontrol agents (Grewal et al., 2005; Laznik et al., 2010; Laznik et al., 2011). That's why they have needed to adapt environmental conditions on application sites to become more effective biocontrol agents (Del Pino et al., 2018).

## **Trapping of entomopathogenic nematodes**

### ***Galleria soil trap method***

The soil trap method is usually used for the extraction of EPNs. 200/250g soil was added into the plastic pots having approximately 90 cm diameter (Razia and Sivaramakrishnan, 2014). Five instar larvae of the greater wax moth, *Galleria mellonella* L. were placed into each pot and covered for incubation of five to seven days at 27-30°C. From the second day onwards, dead larvae were examined and removed regularly. The dead larvae were rinsed with water and dissected into Ringer's solution to collect the entomopathogenic nematodes.

### ***White trap method***

The white trap method is used to collect IJs from cadaver insects. Following this method, dead insects were placed into the white trap for three to four weeks until all juveniles emerged out from the cadaver. Usually, a plastic container was used for this method and filled with distilled water up to 1cm. The bottom of the inverted Petri dish was placed in the container and the juveniles that emerged out were collected (White, 1927).

## **Life cycle**

*Heterorhabditis* and *Steinernema* have similar life stages. These nematodes have a high reproduction rate and can easily culture in lab conditions. They have a chemoreceptor that can detect their host easily and kill them quickly. They are parasitic completely in all stages outside the host except the dauer stage (Onstad et al., 2006). Dauer juvenile is a special developmental stage of all rhabditids. The term dauer is a German word meaning enduring (Fuchs, 1915). It is an infective of these nematodes (Susurluk and Ehlers, 2008). The free-living infective stage is only one stage of their cycle that can exist outside of the host (Spence et al., 2011). Once they find their suitable host they can easily enter into it (Andaló et al., 2017).

Infectious juveniles penetrate the host through the opening including the anus, breathing pore, mouth, spiracles, and pores in cuticles (De Siqueira Sabino et al., 2014) while *S. glaseri* entered into the host through body openings (Hector et al., 2012). Mostly their penetration into the host through breathing pore (Fujimoto et al., 2007).

The relationship between entomopathogenic nematode and entomopathogenic bacteria is highly specific; bacteria *Photorhabdus* spp. and *Xenorhabdus* spp. are associated with *Heterorhabditis* and *Steinernema*, respectively (Ferreira and Malan, 2014). Entomopathogenic nematodes with their symbiotic bacteria can efficiently suppress insect pests in cryptic and soil habitats (Divya and Sankar, 2009). These bacteria entirely depend upon nematodes as a vector from one host to another as well as nematodes immune system of the host (Lewis and Clarke, 2012). The bacteria are rapidly reproduced within the insect and kill it within 24-48 hours, and nematodes are feed on it and complete their life stages (Adams and Nguyen, 2002).

In *Heterorhabditis* infectious juveniles become hermaphroditic adults but their next-generation is produced by males and females while in *Steinernema* all generations are produced by males and females (Grewal et al., 2005). After continuously feeding upon cadaver of insect the second stage juveniles develop into third stage juveniles at that time they leave the insect cadaver for searching new living hosts (Jagdale et al., 2005). The insect cadaver turns into tan or brown if Steinernematids killed it whereas it becomes red when Heterorhabditids killed it, these pigmentations depend upon the mutualistic bacteria (Yadav, 2012).

## Production

Entomopathogenic nematodes are produced by a different method *in vitro* or *in vivo* by liquid and solid culture (Shapiro-Ilan et al., 2012). *In vitro* production, the dauer juveniles are the only stage of entomopathogenic nematode that is used commercially. Culturing is based upon introducing nematodes in a nutrition medium with a pure culture of their symbiotic bacteria. Bedding three-dimensional productions have been the most successful method in solid culture for the production of *Heterorhabditis* and *Steinernema* (Shapiro-Ilan et al., 2012). Large fermentation units are used for the production of these nematodes in large quantities for commercial use.

The liquid culture method is a more cost-efficient process than solid culture for the commercial market. However, it also demands a high level of technical expertise and capital investment (Shapiro-Ilan et al., 2012). Advancement in liquid culture is necessary to improve efficiency and quality of production through different processes like optimizing bioprocess and media kinetics (Chavarría-Hernández et al., 2010), improving inoculum and its timing and density of bacterial cells (Hirao and Ehlers, 2010), improving useful traits such as desiccation and heat tolerance in *Heterorhabditis* and downstream processing (Anbesse et al., 2013).

In *in vitro* liquid culture, EPNs have produced continuously at a high level of efficacy with improvement in media, bioreactor design, and other parameters (Chavarría-Hernández et al., 2010). In *in vivo* production, culturing of EPNs in hosts is a simple process because it requires less technology. *Galleria mellonella* (L.) is the most common insect used for commercial and laboratory EPN cultures whereas *Tenebrio molitor* L. was also used for EPNs production (Shapiro-Ilan et al., 2002). Other hosts have been studied for culturing them, including beet armyworm *Spodoptera exigua* (Hübner), cabbage looper *Trichoplusia ni* (Hübner), corn earworm *Helicoverpa zea* (Boddie), house cricket *Acheta domesticus* (L.), gypsy moth *Lymantria dispar* (L.), orange worm *Ameylois transitella* (Walker), pink bollworm *Pectinophora gossypiella* (Saunders), tobacco budworm *Heliothis virescens* (F.) and various battles (Shapiro-Ilan et al., 2012).

White trap method is used for natural escape of EPNs from a host cadaver. This method is ideal for laboratory studies or small markets because of cost-effective production (Shapiro-Ilan et al., 2002). *In vivo* production is a two-dimensional system based on production in shelves and trays (Ehlers and Shapiro-Ilan, 2005). Its yields depend upon host density and nematode dosage (Boff et al., 2000). *In vitro* production quality may vary from batch to batch (Cottrell et al., 2011). Whereas *in vivo* production depends upon the source of production (Gaugler et al., 2000). Now research is being focused on bioreactor design and media optimization, which expected to lead to benefits such as reduced cost and higher yields (Shapiro-Ilan et al., 2014).

## Storage

Entomopathogenic nematodes can be stored in different ways like water-dispersible granules, autoclaved polyether polyurethane foam, alginate gel, vermiculite and baits. EPNs used less energy because they have no fully dormant resting stage, but juveniles can store a little bit of carbohydrate, protein and lipid in them (Andaló et al., 2009). Their quality depends upon the ratio of viable to non-viable, age, virulence and viability assay (Grewal et al., 2005). Low temperatures up to 2-5°C can increase shelf life and reduced metabolic activity of nematodes except for *H. indica* and *S. riobrave* which cannot survive below 10°C (Grewal, 2002).

## Application

The EPNs can successfully be applied against soil-inhabiting insect pests through soil application and above-ground insects (foliar spray) in cryptic habitats (Shapiro-Ilan et al., 2006). They can be applied through electrostatic sprayers, mist blowers and pressurized sprayers (Shapiro-Ilan et al., 2006), or by mixing them with water dispersal polymers and particular surfactants (Shapiro-Ilan et al., 2010).

## Entomopathogenic nematodes as bio-insecticides

Chemical insecticides are usually used to control pests of fruits, vegetables and crops in China, their extensive use may cause environmental pollution and hamper the export of products (Jianguang et al., 2008). A high frequency of chemical insecticide application may lead to developing resistance in pests (Feng et al., 2000). Chemical insecticides are carcinogenic and cause environmental pollutions because these are not easily degradable.

Entomopathogenic nematodes are used as insecticides (Ulu et al., 2015). They are eco-friendly and safe for human health. Their associations with bacteria have no harmful impacts on other mammals or plants (Ehlers, 2003). The entomopathogenic nematodes were extensively used as pest control agents in various parts of the world (Kaya et al., 2006; Trdan et al., 2008). As compared with chemical insecticides EPNs are too costly for the average grower in China (Yan et al., 2012). Chemical pesticides may cause problems to EPNs if used arbitrarily (Negrisoli Jr et al., 2010). Entomopathogenic nematodes are utilized as augmentative, classical and conservational biological control agents (Lacey and Georgis, 2012). Entomopathogenic nematodes can effectively control the insect pests listed in *Table 1*.

**Table 1.** Insect-pests controlled by entomopathogenic nematodes

Scientific name	Common name	ENP	Reference
<i>Agrotis ipsilon</i>	Black cut worm	<i>H. amazonensis</i>	(de Oliveira Giannasi et al., 2018)
<i>Alissonotum impressicolle</i>	Banana borer	<i>H. indica</i>	(Anh et al., 2017)
<i>Anomala graueri</i>	White grub	<i>S. longicaudum</i>	(Kajuga et al., 2018)
<i>Anoplophora glabripennis</i>	Long-horn beetle	<i>S. carpocapsae</i>	(Solter et al., 2001)
<i>Arbela dea</i>	Litchi beetle	<i>S. carpocapsae</i>	(Saleh, 2017)
<i>Bactrocera tryoni</i>	Queensland fruit fly	<i>H. bacteriophora</i>	(Langford et al., 2014)
<i>Bactrocera zonata</i>	Peach fruit fly	<i>H. marelatus</i>	(Saleh et al., 2018)
<i>Bradysia odoriphaga</i>	Chive maggot	<i>H. bacteriophora</i>	(Bai et al., 2016)
<i>Carposina nipponensis</i>	Apple fruit moth	<i>S. carpocapsae</i>	(Yang et al., 2000)
<i>Cephus cinctus</i>	Wheat stem sawfly	<i>S. kraussei</i>	(Portman et al., 2016)
<i>Chilo infuscatellus</i>	Sugarcane borer	<i>S. feltiae</i>	(Karunakar et al., 2002)
<i>Chironomus plumosus</i>	Buzzer midge	<i>S. kraussei</i>	(Edmunds et al., 2017)
<i>Coptotermes formosanus</i>	Termite	<i>S. kari</i>	(Wagutu, 2017)
<i>Curculio elephas</i>	Chestnut weevil	<i>S. weiseri</i>	(Demir et al., 2015)
<i>Cydia pomonella</i>	Codling moth	<i>S. jeffreyense</i>	(Odendaal et al., 2016)
<i>Earis vittella</i>	Spotted bollworm	<i>S. mushtaqi</i>	(Pervez and Ali, 2011)
<i>Ectomyeolis ceratoniae</i>	Carob moth	<i>S. carpocapsae</i>	(Memari et al., 2016)
<i>Eriosoma lanigerum</i>	Woolly apple aphid	<i>H. megidis</i>	(Berkvens et al., 2014)
<i>Heliolithis virescens</i>	Tobacco budworm	<i>H. bacteriophora</i>	(Gulzar et al., 2020)
<i>Holocercus insularis</i>	Tree borer moth	<i>S. carpocapsa</i>	(Kaya et al., 2006)
<i>Holotrichia consanguinea</i>	Sugarcane beetle	<i>S. abbasi</i>	(Patil et al., 2016)
<i>Holotrichia oblita</i>	White grub	<i>S. longicaudum</i>	(Guo et al., 2015)
<i>Holotrichia parallela</i>	Peanut grubs	<i>H. beicherriana</i>	(Li et al., 2021)
<i>Hylobius abietis</i>	Large pine weevil	<i>S. downesi</i>	(Kapranas et al., 2017)
<i>Leucinodes orbonalis</i>	Brinjal fruit borer	<i>S. siamkayai</i>	(Adiroubane et al., 2010)
<i>Maconellicoccus hirsutus</i>	Mango mealybug	<i>H. amazonensis</i>	(Fuenmayor et al., 2020)
<i>Macrotermes bellicosus</i>	Termite	<i>H. sonorensis</i>	(Zadji et al., 2014)
<i>Mamestra brassicae</i>	Cabbage moth	<i>S. carpocapsae</i>	(Beck et al., 2014)
<i>Musca domestica</i>	Housefly	<i>H. indica</i>	(Bream et al., 2018)
<i>Mythimna separate</i>	Armyworm	<i>H. indica</i>	(Acharya et al., 2020)
<i>Odoiporus longicollis</i>	Banana borer	<i>S. carpocapsae</i>	(Belien, 2018)
<i>Otiorynchus ligustici</i>	Alfalfa snout beetle	<i>H. bacteriophora</i>	(Shields et al., 2009)
<i>Paranthrene diaphana</i>	Clearwing moth	<i>S. carpocapsae</i>	(Azarnia et al., 2018)
<i>Periplaneta americana</i>	Cockroach	<i>S. carpocapsae</i>	(Maketon et al., 2010)
<i>Phyllotreta cruciferae</i>	Crucifer flea beetle	<i>S. carpocapsae</i>	(Reddy et al., 2014)
<i>Phyllotreta striolata</i>	Striped flea beetle	<i>S. feltiae</i>	(Xu et al., 2010)
<i>Planococcus ficus</i>	Vine mealybug	<i>S. yirgalemense</i>	(Platt et al., 2018)
<i>Plutella xylostella</i>	Diamondblack moth	<i>S. carpocapsae</i>	(Sunanda et al., 2014)
<i>Polyphylla fullo</i>	Pine chafer	<i>S. glaseri</i>	(Demir et al., 2015)
<i>Rhynchophorus ferrugineus</i>	Red palm weevil	<i>S. carpocapsae</i>	(Dembilio et al., 2010)
<i>Scirpophaga incertulas</i>	Rice stem borer	<i>H. bacteriophora</i>	(Devi, 2020)
<i>Spodoptera littoralis</i>	Cotton leaf worm	<i>S. monticolum</i>	(Sobhy et al., 2020)
<i>Spodoptera litura</i>	Armyworm	<i>S. glaseri</i>	(Safdar et al., 2018)
<i>Stomoxys calcitrans</i>	Stable fly	<i>H. baujardi</i>	(de Souza Leal et al., 2017)
<i>Tuta absoluta</i>	Tomato leaf miner	<i>H. bacteriophora</i>	(Damme et al., 2016)
<i>Zeuzera pyrina</i>	Leopard moth	<i>H. bacteriophora</i>	(Salari et al., 2015)

Whereas; ENP (entomopathogenic nematodes). List of insect pests currently controlled by applications of entomopathogenic nematodes

## Mechanisms of EPNs

Entomopathogenic nematodes are deadly insect parasites that generate and discharge toxins into their host's body. Main venom proteins include both immune-modulating and tissue-damaging proteins, that these parasites have both a general and a specialized group of effectors, and a modified collection that is more unique to the hosts they invade (Chang et al., 2019). They are used as templates for host-parasite relationships such as host searching behaviour (Lewis et al., 2006), triggering of the parasite (Alonso et al., 2018),

the function of secreted-products in parasitism (Lu et al., 2017), and ecology (Hodson et al., 2012). Numerous experiments revealed that juveniles of *S. feltiae* use their cuticle to block the immunity of the host (Brivio et al., 2004). Helminthes are commonly known as modulating the host's immune system and inducing pathology primarily by the secretion of small molecules and proteins that interfere with the host's cells (Brivio et al., 2004).

### ***Secretion of lethal venom***

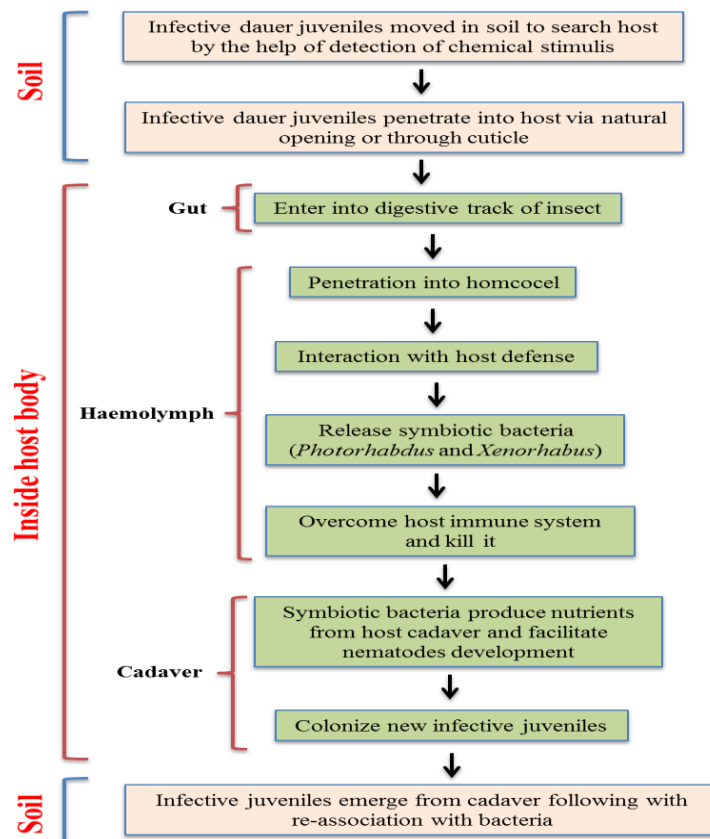
Entomopathogenic nematodes have been widely believed to serve mostly as a vector for pathogenic-bacterial symbiosis. Besides, when these bacteria enter the host, they start to multiply and feed on the tissues of the host, which is responsible for the death of the host (Karthik et al., 2014). There is, however, an increasing body of investigation identifying nematode as a key asset to pathogenesis and in certain instances such as *S. scapterisci* play the role of the key agent of virulence (Lewis and Clarke, 2012). Apart from acting as a conduit for the bacteria they bring, it is evident that EPNs aid pathogenesis in two different ways such as; they actively destroy the tissue and impede the defense of the host, allowing more energy for themselves and the bacteria they contain to conquer and abolish the host. Previous investigations have revealed that axenic juveniles of *S. carpocapsae* can multiply within the host and destroy the host (Han et al., 2000; Sicard et al., 2003). Specific Steinernematidae effector molecules have been described and shown to participate in tissue damage and immune suppression of host (Toubarro et al., 2013a,b). Chang et al. (2019) revealed that both *S. carpocapsae* and *S. feltiae* possess a greater concentration of excreted/secreted proteins (ESPs) which are either Ig-like, Von Willebrand, or Ig (immunoglobulin) and FAR (fatty acid/retinol binding-protein). Excreted/secreted proteins (ESPs) are the primary link between hosts and parasites and therefore impact the protection of the parasites and their toxicity of the hosts (Cuesta-Astroz et al., 2017). However, FAR proteins were believed to influence immune signaling (Kennedy et al., 2013). These secretions of EPNs were found to be a complicated mixture comprising several proteins and together, it is poisonous to insects (Chang et al., 2019).

### ***Nematode defenses and the immune system of insect***

Nematode parasites and their related bacteria are ideal pathogenic agents for anti-bacterial and anti-nematode immune responses to insect host searches (Kenney and Eleftherianos, 2016). The nematode immune-modulation mechanism demonstrates the variety of modulatory strategies developed for suppression of host immune response by various types of parasitic nematodes (Cooper and Eleftherianos, 2016). In a study, Toubarro et al. (2013b) reported that *S. carpocapsae* displayed destructive approaches for host immunity through proteolytic secretion which inhibits host immunological defenses.

The nematodes and bacteria collaborate to suppress the immune response of the host, allowing vegetative replication of the bacteria (Dowds and Peters, 2002). *Xenorhabdus nematophila* and *S. carpocapsae* can inhibit the antibacterial peptide immune reaction of insects (Binda-Rossetti et al., 2016). The molecular studies of various insect hosts showed that both *Heterorhabditis* and *Steinernema* have a wide variety of results. Such as, *Manduca sexta* and *S. exigua*, impedes the transcription genes of insect those encoding for antimicrobial peptides (AMPs); in *X. nematophila* and *S. exigua*, cells were capable to impede the development of nodules by inhibiting the biosynthetic pathway of eicosanoid while in *P. luminescens* and *M. sexta*, cells released an anti-phagocytic factor that enabled the bacterial cells to disrupt their phagocytosis (Silva et al., 2002; Park et al., 2003, 2007).

Both bacteria *Photorhabdus* and *Xenorhabdus* have also been displayed similar lifestyles but have different molecular defensive mechanisms (Goodrich-Blair and Clarke, 2007). The symbiotic *Xenorhabdus* bacterium inhibits the host's immune system by producing a variety of toxins and carrying type III effector molecules that may interfere with the actin cytoskeleton and prevent phagocytosis (Dillman et al., 2012a). *Photorhabdus* used lipopolysaccharide (LPS) modification to resist the action of the host-derived AMPs (Eleftherianos et al., 2006), while *Xenorhabdus* prevents induction of insect AMP expression altogether (Istkhar et al., 2019). In insects, the pathogenic impacts of bacteria and the anti-bacterial resistance mechanisms have been well described, however, nematode-associated defenses are nowadays primarily the focus of research. The EPN-pest interaction is illustrated in *Fig. 1*.



**Figure 2.** The interaction between entomopathogenic nematodes and pests

### Commercially available entomopathogenic nematodes worldwide

In recent years, *Heterorhabditis* and *Steinernema* products have successfully been manufactured and commercialized by various companies around the world. The most popular species that have been successfully manufactured and utilized as commercial products are *H. bacteriophora*, *H. downesi*, *H. indica*, *H. megidis*, *S. carpocapsae*, *S. feltiae*, *S. kraussei*, *S. kushidai*, *S. riobrave* and *S. scapterisci* (Table 2). The EPN products are marketed under various brands, in developed and developing countries of the world, such as Austria, Australia, Belgium, Canada, Germany, India, Japan, Kenya, Lietuva, Netherlands, Newzealand, Poland, the United Kingdom and the United State of America.



**Table 2.** Commercial products of entomopathogenic nematodes (EPNs) prepared by different countries

EPN	Commercial product	Manufacturer
<i>H. bacteriophora</i>	Larvanem NemaShield-HB Nematop NemaTrident-C Nema-green Otinem Optinem-H E-Nema GmbH	Koppert Biological System, Berkel en Rodenrijs, NL BioWorks Inc. Victor, New York State, US BioForce Limited, Drury, NZL Bionema Limited, Swansea, UK e-nema, Schwentidental, Germany Bioenterprises PTY Limited, Roseville, NSW Agronovos, Ažuolo g. 25, Alionių II k, Lietuva e-nema, Schwentidental, Germany
<i>H. downesi</i>	NemaTrident-CT	Bionema Limited, Swansea, UK
<i>H. indica</i>	Calterm Grub Terminator GrubStake-Hi Soldier	Kisan Manch, IN Benzer Crop Science, Sirsi, IN Integrated Biocontrol System Inc., Greendale IN Swami Samarth Agro Biotech LLP, Pune, IN
<i>H. megidis</i>	LarvaNema Nemasys H	Koppert B.V., Berkel en Rodenrijs, NL MicroBto, Cambdng, UK
<i>S. carpocapsae</i>	BioSafe Biosafe-N BioVector Bouncer Boden-Niützlinge Capsanem Carpocapsae-System Exhibitline SC Helix Optinem-C Mioplant NemaGard Nemastar NemaTrident-T Nemasys C Palma-Life Vector TL X-GNAT	SDS Biotech, Minato-Ku, Tokyo, Japan Thermo Triology, Corp., Columbia, MD Thermo Triology, Corp., Columbia, MD Swami Samarth Agro Biotech LLP, Pune, IN Rhone-Poulenc, Cellaflor, Germany Koppert Biological System, Berkel en Rodenrijs, NL Biobest Sustainable Crop Management, Westerlo, BEL Bioline AgroSciences Ltd. Camarillo, US Novartts, Misnssauga, Canada Agronovos, Ažuolo g. 25, Alionių II k, Lietuva Novartts, Vienna, Austria Purely Organic Products LLC, Portsmouth, US BioForce Limited, Drury, NZL Bionema Limited, Swansea, UK BASF Corporation, Ludwigshafen, Germany Biobest Sustainable Crop Management, Westerlo, BEL Lesco, Lansing, MI E C Geiger, Harleysville, PA
<i>S. feltiae</i>	Entonem Exhibit Magnat Nemasys NemaShield NemaTrident-F Nemapom Nemaplus Nemaflor Nemasys F Nematech-S SP NemaTrident-S Nemax-F Nemycel Optinem-F Owinema SC Stealth	Koppert Biological System, Berkel en Rodenrijs, NL Novartts, Basel, Switzerland Amycel-Spawn Mate, Watsonville, CA MicroBto, Cambdng, UK BioWorks Inc. Victor, New York State, US Bionema Limited, Swansea, UK e-nema, Schwentidental, Germany BioForce Limited, Drury, NZL e-nema, Schwentidental, Germany BASF Corporation, Ludwigshafen, Germany Dudutech, Naivasha, Kenya Bionema Limited, Swansea, UK Serbios, Badia Polesine, Italy e-nema, Schwentidental, Germany Agronovos, Ažuolo g. 25, Alionių II k, Lietuva Owiplant Ltd. Horticultural Enterprise, Poznań, PL Novartis, Macclesfield, Chester, UK
<i>S. kraussei</i>	Exhibitline Sk	Bioline AgroSciences Ltd. Camarillo, US
<i>S. kushidai</i>	SDS biotech Kraussei-System	SDS Biotech K.K, Tsukuba, Japan Biobest Sustainable Crop Management, Westerlo, BEL
<i>S. riobrave</i>	Biovector Vector MC	Thermo Triology, Corp., Columbia, MD Lesco, Lansing, MI
<i>S. scapterisci</i>	Proactant Ss	BtoControl, Gamesville, FL

The representative trade names are those displayed on the respective company websites

## Conclusion

In the end, it is concluded that EPNs are suggested to be used as biopesticides due to huge host range, safety and compatibility within a variety of environmental conditions. Entomopathogenic nematodes are excellent biological control agents for soil-dwelling insects. Its mutual association with bacteria can easily kill insect pests so that they can be applied easily. Better understanding and development of EPNs are necessary for a suitable replacement of synthetic pesticides. More research is needed in China and all over the world to evaluate the biopesticides potential of EPNs as an effective tool for integrated pest management (IPM). Nowadays, the immune system of insects is being studied on a wide scale but their associations with EPNs are still less studied. The analysis of insect defenses and EPNs offenses would give a more precise description for effective control of insect pests as a future prospect. A better understanding of insect defense mechanisms against the EPNs and decreasing them by some adjuvants or nematode species with complex pathogenicity and powerful immune-suppressive abilities may be useful for potential pest management programs in the future.

**Acknowledgements.** The financial support was provided by the National Nature Science Fund Key Projects (313300630), China Agriculture Research System CARS-04-PS13 and National Parasitic Resources Center (NPRC-2019-194-30).

**Conflict of interests.** Authors have no conflict of interests.

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