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REVIEW ARTICLE

A Review on Entomopathogenic Nematodes: *Heterorhabditis* and *Steinernema*

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ABSTRACT
Insecticide resistance and environment threat due to injudicious use of chemical pesticides for insect pest management employs the introduction of a new alternative as biological control. Entomopathogenic nematodes possessing reasonable biological control attributes belongs to genera Heterorhabditis and Steinernema. They have mutualistic association with bacterial genus Photorhabdus and Xenorhabdus respectively. Entomopathogenic nematodes are highly effective to soil born insect pests and are safe to nontarget organisms. Pathogenic effect of entomopathogenic nematode and their symbiotic bacteria kill the insects within 24-48 hours as compared to days and weeks required for insect killing by other biological control agents. Entomopathogenic nematodes are lethal to insects, motile, self perpetuating in field, tolerate short time exposure to agrochemicals and easy to mass multiply, necessitate its exploration against a number of insects of order Homoptera, Diptera, Coleoptera and Lepidoptera. EPN are also compatible with fertilizers, biological insecticides, fungicides, herbicides, and growth regulators, thus providing an opportunity of application together. This review article facilitates the researchers to overview of the work done and move forward related to different aspects of entomopathogenic nematode.
Keywords: *Entomopathogenic nematodes (EPN), Biological control, Steinernema. Heterorhabditis*

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INTRODUCTION

Indian economy is based on agriculture as more than 60% of its population directly or indirectly dependent on agriculture which contributes nearly 17.9% to country's GDP [1]. India would require more than 450 million tons of food grains to fulfill the requirement of 1.65 billion people by 2050 which will be a very difficult task [2]. The substantial increase in food grain production over the years has helped to meet the food security needs of the country, but the number of biotic and abiotic stress causes the yield losses up to a large extent. Estimated crop losses caused by insect pests to major agricultural crops in India is about 17.5% and in monetary terms the value is 86, 3884 million rupees [3]. Therefore pesticide consumption was also increases year by year as 45.39 thousand tons pesticides were consumed in the year 2012-13 and 65% of them were shared by insecticides [4]. Besides affecting the environment and non targeting organisms, continuous and tremendous use of chemical pesticides creates high selection pressure on pest population which force to mutation inside the insects and development of pesticide resistance in insects pest. Biological control is a key constituent of integrated pest management that has generated interest among farmers for ecological and sustainable insect pest management. Detailed study of these Entomopathogens can direct to useful exploitation in biological control programmes of Integrated Pest Management.

Nematodes are microscopic roundworms, colorless, pseudosegmented, and without appendages, having pseudocoelome, may be free-living, parasitic or predaceous. It may be pathogenic to animal, human and plants. Entomopathogenic nematodes (EPNs) are pathogenic to insects therefore can be used as a component of integrated pest management against many insect pests. Seven families of nematode include species that have potential for the use as EPN [5]. Only few families have potential of insect killing but

some of them are difficult to mass production (tetradomatids), have narrow host specificity or have modest virulence (e.g., sphaeruliids). EPNs possessing optimal biological control properties are only in the *Steinernema* and *Heterorhabditis* genera.

Habitat:

Steinernematids and Heterorhabditids are exclusively soil organism. They are cosmopolitan, having been isolated from ecologically diverse soil habitats including crop fields, gardens, forests, grasslands and deserts. Soil property such as soil texture, type, bulk density, organic content, soil water potential and pH can affect Infective juvenile behavior, survival, and infectivity to hosts. Anaerobic condition in water-saturated soils and soils with contents of organic matter may become a limiting factor. High bulk density can impede nematode migration since soil porosity can be too narrow to allow efficient movement [6]. Steinernematids are ambusher in nature so they are present on upper surface of soil while Heterorhabditids are cruiser i.e. they may present on deeper layer of soil.

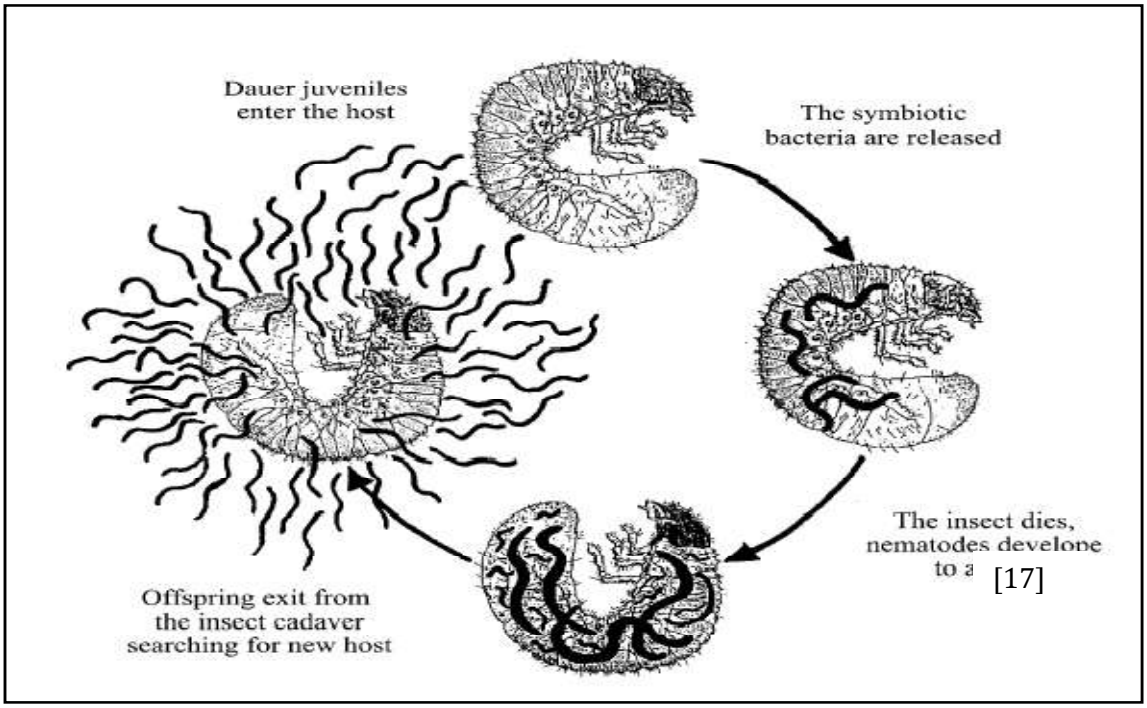
Mutualistic bacteria:

Photorhabdus and *Xenorhabdus* both genera of bacteria shows mutuallistic relationship with *Heterorhabditis* and *Steinernema* genera of nematode respectively and pathogenic relationship with host insect [7]. Both bacteria are belonging to the family Enterobacteriaceae they are motile (Peritrichous flagella) nonendospore froming, facultative anaerobe, gram negative rod. They secrete a variety of toxins, antifungal, antibacterial agents and hydrolytic enzymes such as lipases, phospholipases, chitinases, luciferase and proteases [8]. Nematodes relies on bacteria for killing its insect host, creating suitable environment for its development by producing antibiotics that suppress competing secondary microbes, breakdown of host tissue in to usable nutrients and to serve as food source. Bacterium required nematodes for protection from external environment, penetration into host haemocoel, and inhibition of host's antibacterial protein. Antimicrobial compounds of *Xenorhabdus* and *Photorhabdus* are Anthraquinones, Macrolides, Nucleosides, Hydroxystilbenes [9]. Genitein, Nematophein [10], Indole, Xenoxides, Xenorhabdins, Xenocoumacins [11].

LIFE CYCLE AND MODE OF ACTION

The free-living, non-feeding, nonexcrteory and developmentally ceased juvenile stage 3 of both the genera (*Heterorhabditis* and *Steinernema*) is act as infective juvenile (IJ3), they are motile having chemoreceptors, high reproductive potential like pathogens, highly virulent, kill their hosts rapidly, and easily culturable. When a host has been located with the help of chemoreceptors or by other means, the nematodes penetrate into the insect body, usually via natural body openings (mouth, anus, spiracles) or areas of thin cuticle i.e. intersegmental membrane. Multitrophic interaction occurs between the Nematode-Bacteria and Insect. The infective juveniles can penetrate directly through the cuticle or indirectly via gut to enter the haemocoel. To enter through the cuticle, the nematodes utilize physical force such as to-and-fro movement by thin trachea. *Heterorhabditis*, use an anterior tooth to penetrate directly into the haemocoel. To enter through the gut, they use physical force and/or hydrolytic enzymes to digest the midgut tissues to gain access into the hemocoel [12]. Within the insect's haemocoel, the nematodes and bacteria overcome the host's immune response [13].

However, many humoral and cellular factors are involved to counteract the nematodes such as Insect may use antibacterial proteins, enzymes and/or phagocytosis followed by nodulation, and the insect's haemocytes may encapsulate the nematodes followed by melanization. Sometimes the nematodes can overcome the insect defenses. Thus, *S. glaseri* is primarily encapsulated by larvae of the *Popillia japonica*, but it escapes from the capsule and successfully infects its host [14] because the nematode has surface coat proteins that suppress the host's immune response and destroy the haemocytes [15]. A *Heterorhabditis* species avoids encapsulation in tipulid larvae by removing the sheath from the second-stage cuticle during host penetration [16].



[17]

Fig. 1 Life cycle of Entomopathogenic Nematode

Insect behaviors such as high defecation (scarab grubs), low CO_2 release (lepidopterous pupae and scarab grubs), formation of impenetrable cocoons (many lepidopterans and scarabs), walling-off nematode killed individuals that avoid contamination to other insects in a nest (termites), and aggressive grooming and leaking behavior that reduces infective juvenile contact (scarab grubs) [18]. In some insects, the usual routes of entry may be difficult to get due to hindrance created by Oral filters (wireworms), too narrow mouth opening (insects with piercing and sucking mouthparts), anus may be constricted by muscles (wireworms), or spiracle opening may be covered with septa (wireworms) and by sieve plates (scarab grubs). Moreover, the invading nematodes can produce immune-suppressor that destroy the antibacterial factors produced by the insect and facilitate the mutualistic bacteria to produce variety of insecticidal toxins, antifungal, antibacterial agents and hydrolytic enzymes such as chitinases, lipases, phospholipases, luciferase and proteases [8,19,20] that quickly kill the host within 24-48 hours. Nematodes also produce paralyzing exotoxins and cytotoxic and proteolytic enzymes. The above phenomenons are dependent on the tritrophic interaction among the insect host, nematode and associated bacteria which contribute to the variable efficacy of EPNs against different insect species.

Table no. 1 List of host insects targeted by entomopathogenic nematodes

Order	Common name	Scientific name	Crop	Nematode spp.	References
Diptera:					
Sciaridae	Fungal gnats	*Bradysis spp*	Green house mushrooms	*S. feltiae*	[21]
Tephritidae	Mediterranean fruit fly, Peach fruit fly	*Ceratitis capitata, Bactrocera zonata*	Fruit pest	*H.bacteriophor, S.feltiae,*	[22]
Lepidoptera					
Tortricidae,	Codling moth	*Cydia pomonella*	Soil	*H. zealandica*	[23]
Pyralidae	Oriental fruit moth	*Grapholita molesta*	Peach	*S.carpocapsae, S. feltiae.*	[24]
Sesiidae	Peachtree borer	*Synanthedon pictipes*	Stone fruits	*S. pictipes* and *S. carpocapsae*	[25]
Tortricidae,	Filbertmoth	*Melissopus Latiferreanus*	Hazelnuts	*S. carpocapsae*	[26]
Noctuidae	Black Cutworm	*Agrotis ipsilon*	Turf grass	*H.bacteriophor, S.carpocapsae*	[27]
	Tobaccocattterpillar, Tomato fruit borer	*Spodoptera litura, Helicoverpa armigera*	Foliar crops	*H. indica* and *S. glaseri*	[28]

Plutellidae	Diamond Back Moth (DBM)	*Plutella xylostella*	Cabbage	*H. indica* and *S. glaseri*	[28]
Pieridae	Cabbage butterfly	*Pieris rapae*	Cabbage	*H.bacteriophor*	[29]
Gelechiidae	Potato tuber moth	*Phthorimaea operculella*	Potato	*H. idica* and *S bacteriophora*	[30]
	Tomato leaf miner	*Tuta absoluta*	Tomato	*S. feltiae* and *H.bacteriophor*	[31]
Coleoptera:					
Scarabeidae	White Grub	*Holotrichia parallela*	Peanut	*S. longicaudum*	[32]
Chrysomeliae	Stripped beetle	*Acalymma vittatum*	Cucurbit	*S. riobravis*	[33]
Thysanopter					
Thripidae	Thrips	*Frankliniella occidentalis*	Vegetable, Ornaments	*H.bacteriophoa, S. feltiae*	[34]

Mass production

Entomopathogenic nematodes are currently produced by two methods as *in vivo* or *in vitro* (solid and liquid culture) [35].

***In vivo* production:**

Production methods for culturing entomopathogenic nematodes in insect hosts have been reported by many authors [36]. Culturing of EPN through *in vivo* method requires a live insect hosts. This method employs minimal technology and involves the use of a surrogate host insect. The most common insect host used for *in vivo* laboratory and commercial EPNs production is the late instar larvae of greater wax moth, *Galleria mellonella*, [37]. It is based on the White trap method [38], which utilizes natural movement of infective juveniles (IJ) as they move away from the host cadaver when nutrient resources get depleted. Yield of IJs is depends on choice of nematode and insect host species. Generally yield of nematodes is proportional to size of insects [39,40] but susceptibility to infection is negatively correlated with host size or age [39]. Therefore mature insect is less susceptible as compare to larval stage. This method is not much cost effective for large scale industry [37] although it can be ideal for cottage industry or for laboratory study.

***In vitro* production:**

In vitro culturing of entomopathogenic nematodes is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium. The only stage that can be commercially used is the dauer juvenile (DJ) which is a nonfeeding and nonexcreating stage and morphologically distinct, formed as a response of food scarcity and adverse environmental conditions. Mass production of entomopathogenic nematodes has started from the first large scale *in vitro* solid media production by Glaser and coworkers [41] to the three dimensional solid media (media suspended in foam) *in vitro* process by Bedding [42]. Wout's medium, wheat flour medium, dog biscuit medium, egg yolk medium can be used in solid media process but wouts media is most cost effective [43]. Yield of IJs depends on ingredient of culture media such as lipid, protein salt and carbon sources. Lipid components act as nematode's natural host composition and are most suitable [44]. Increasing the lipid quantity and quality leads to increases in nematode yield [45]. Solid media process of *Steinernema* and *Heterorhabditids* production is more advantageous for small scale industry. First attempts to culture EPN in liquid media were made by Stoll [46] to the *in vitro* liquid fermentation production method [47]. Liquid fermentation process required maintenance of oxygen level and removal of CO_2 and toxic gases for the multiplication of EPNs. In liquid fermentor process symbiotic bacteria are first introduced followed by the nematodes [48]. Components for liquid culture media have been reported as soy flour, yeast extract, canola oil, corn oil, thistle oil, egg yolk, casein peptone, milk powder, liver extract and cholesterol [49,50]. Culture times vary depending on media and species, and may be three weeks [51]. Once the culture is completed, nematodes can be harvested from media via centrifugation [49]. Liquid-fermentation process is highly efficient for the production of several *Steinernema* species but not suitable for *Heterorhabditids* [52] and useful for large scale industry. IJs yields in liquid culture depend on the degree of recovery which can be affected by nutritional factors, aeration, CO_2, lipid content, and temperature [50].

Formulation and storage:

An important prerequisite for successful application of an antagonist in biological control is its stable commercial formulation. A variety of formulations have been developed to facilitate nematode storage and field application including charcoal, alginate and polyacrylamide gels, baits, clay, peat, polyurethane sponge, vermiculite, and water-dispersible granules [53]. Active nematodes must be immobilized for the

stability of their lipid and glycogen reserves. Formulated entomopathogenic nematodes can be stored for 2 to 5 months depending on the nematode species and storage media and conditions. Low storage temperature (2-7^{0}C) generally suspended metabolic activity and therefore enhances their shelf-life [54]. However some warm adapted species such as *H. indica* and *S. riobrave* do not store well at temperature less than10ºC [55]. The quality of the nematode product can be determined by nematode virulence and viability assays, age, and the ratio of viable to non-viable nematodes [56].

Table no. 2 Commercial products available in international market

Nematode species	Product formulation	Country
Steinernema carpocapsae	Biosafe, Biovector	USA
	Sanoplant	Switzerland
	Boden Nutz;linge	Germany
	Helix	Canada
	Green commandos, Soil commandos	India
S.feltiae	Nemasys	UK
	Entonem	USA
	Nemaplus	Austria
S. riobrave	Vector MG	USA
Heterorhabditis megidis	Nemasys	UK
	Larva nema	USA
H.bacteriophora	Cruiser, Nematop, Nema green	USA

Application and factors affecting efficacy:

In some instances, EPNs have proven to be ecofriendly and effective alternatives of chemical pesticides, but in several other cases they have failed to compete successfully with chemical pesticides [53]. Insufficient results of entomopathogenic nematodes as pest control agents are caused by improper handling, transport, and storage [57]. The nematode efficacy can be enhanced by development of new technology in nematode production, formulation, quality control, storage, application timing and delivery, and mainly in selecting target pests and habitat. Entomopathogenic nematodes are living organisms, and affected by both biotic and abiotic factors during applications. Abiotic factors such as temperature, humidity, soil pH, UV light, dessication etc. Entomopathogenic nematodes work best in sandy soil with a pH between 4 and 8 [37]. *S. ribrave, S. glaseri,* and *H. indica* are heat tolerant species while *S. feltiae, H. megidis* and *H. marelatus* are adapted to cooler temperature [58]. All biological control agents including nematodes require a specific condition for their effectiveness. Desiccation and high temperature are the most important abiotic factors affecting survival of EPNs [59]. Nematodes have limited temperature range (20 °C and 30 °C) for their effectiveness. Treated area should be kept moist for at least 2 weeks [60] to avoid desiccation. In soil, infective juveniles are attacked by a number of biotic factors such as pathogens or predators viz. phages, bacteria, protozoans, nematophagous fungi, predacious mites and nematodes, etc. [61] and act as antagonist of EPNs while *Paenibacillus popilliae* [62] and *Bacillus thuringiensis* [63] work synergistically with EPNs.

The most commonly used application method for entomopathogenic nematodes is spraying directly on to the field. 1 billion nematodes per acre is recommended rate for the broadcast application of EPNs to control most soil insects [53]. Entomopathogenic nematodes can be applied with nearly all agrochemical equipments of agriculture and horticulture ground including hand or ground sprayers, mist blowers, and electrostatic sprayers or as aerial sprays [64]. The infective juveniles can withstand pressure up to 1068 kPa and pass through all common nozzle type sprayers with openings of about 100 µm in diameter [53] in diameter. Rate of nematode application can be increased or decreased according to target pest and conditions but generally 25 infective juveniles per cm^2 are recommended dose for the nematode application but it can be increased or decreased depending upon target pest [58].

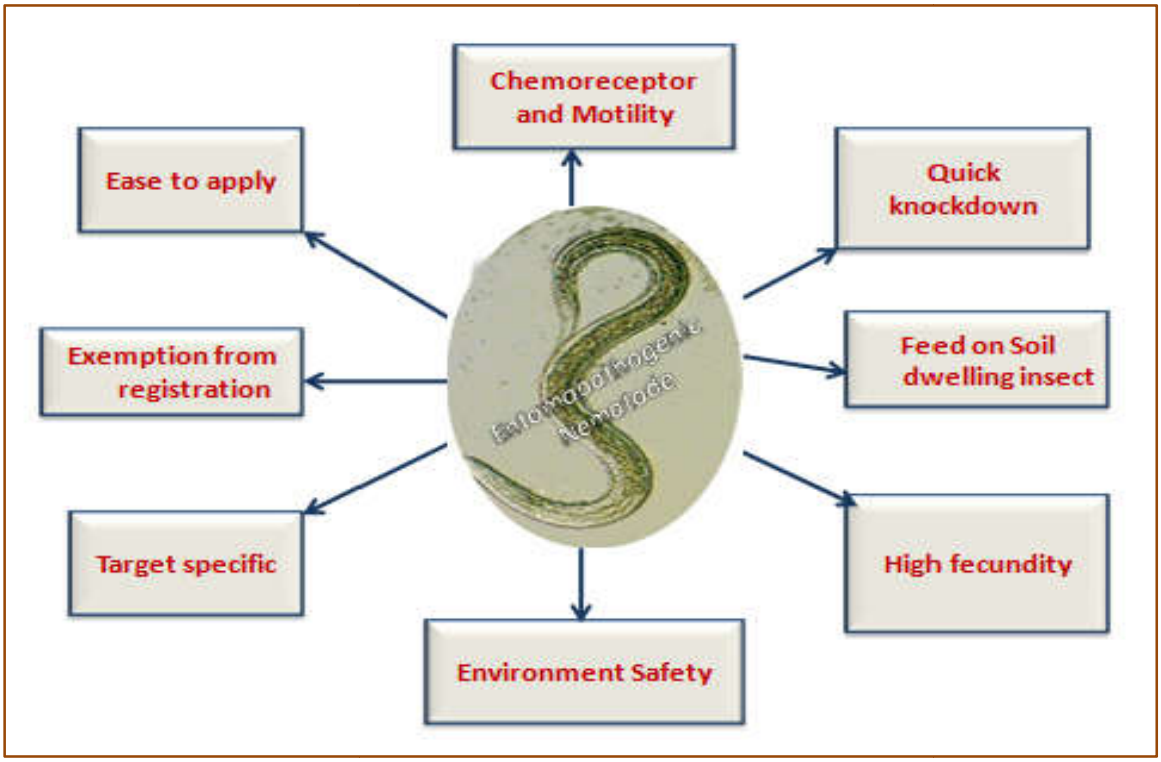


Fig. 2 Benefits of Entomopathogenic Nematodes

CONCLUSIONS

Entomopathogenic nematodes have great potential to use as biological control agent against insect pest especially for soil dwelling insects in integrated pest management programme. Host seeking capability, quick knockdown, exemption from registration, ease of application along with ease of production, high fecundity and environment safety are attributes which makes the Entomopathognic nematode special and exclusive from many other Biological control agents. They also have a broad host range, exhibit synergistic effect with other control agents and are compatible with some agrochemicals for short time exposure. Many developed countries such as USA, Canada, Australia, Germany, UK etc. have excellent marketability in international market. The opportunities for using EPNs against insect pests in the soil and cryptic habitats in India are also tremendous. Recent emphasis is needed for the development of advance technology with special emphasis on mass production; storage and formulation are required to implement safer and effective pest control methods. Research is required to work in the area of factors which regulate their population dynamics and on how their population can be manipulated to enhance the epizootic in insect-pest populations. Finally, apart from their use as biopesticides they also contribute to science for understanding the evolution of symbiosis and parasitism as tritrophic interaction between Nematode-Bacteria-Insect.

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