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12. Entomopathogenic Nematodes: An overview

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Abstract:

The term "entomopathogenic" (or "insect-pathogenic") nematodes refers to a group of soil-dwelling roundworms that prey on insects that reside in, on, or near the soil surface and are typically intimately related to plants. The term "beneficial" or "insecticidal nematodes" refers to these tiny parasitic roundworms, which attack and frequently quickly kill underground insects. The phylum Nematoda contains species of namatodes from the genera Steinernema and Heterorhabditis. EPN can be found in a variety of natural settings, such as deserts, farmed fields, forests, grasslands, and coastal beaches. The finest biological control agents for many insect pests' soil-dwelling stages can be found in them, and they work quickly to eliminate their target pests in 24 to 48 hours. Despite being completely safe for plants and animals, EPN are incredibly devastating to many significant soil insect pests.

Keywords:

Nematode, Management, EPN, insect – pest, Sustainable farming.

12.1 Introduction:

Nematodes are a group of thread or worm like, transparent, bilaterally symmetrical with external cuticle, pseudocoelomate and multicellcular organisms, that are free living (in soil or water) or parasitic to plants / animals (Abd-Elgawad, 2017). Based on the feeding habits nematods may be classified into three main groups

- **Saprophagous:** living, on dead organic matter of plant and animal origin and micro – organisms associated with decay.
- **Predators:** Feeding on small animals including namatodes.
- **Parasites:** infe-sting insects, animals, man, fungi and higher plants.

Nematodes that have affinity towards insects are known as Entomophilic nematodes. The other nematodes species which associate or attack insects are called as Entomognous nematodes while entomopathogenic nematodes parasites and kill insects and may serve as bio control agents (Baiocchi *et al*., 2017; Abd-Elgawad, 2019). Entomopathogenic nematodes are a suitable fit for integrated pest management programmes because they are generally specific to the pests they target, are assumed to be non-toxic to humans, and can be applied with standard pesticide equipment (Shapiro-Ilan *et al*., 2006).

Entomopathogenic namatodes are not required to register as pesticides with the US Environmental Protection Agency (EPA). Personal protective equipment and re-entry restrictions are not necessary (Dolinski *et al*., (2015). Problems with insect resistance are unlikely. Nematodes, acting with their symbiotic bacteria, kill insect - pest, in one or two days, but the majority of bio-control agents take days or weeks (Abd-Elgawad, 2020). Numerous different insect pests are vulnerable to infection, although no unfavorable effects have been observed against non-target organisms under field conditions (Georgis *et al*., 1991; Bal *et al*., 2014). Nematodes can be applied using normal agricultural-chemical equipment such as pressurized mist, electrostatic fans, and aerial sprayers; they don't need specialized equipment for this. Nematodes belong to the families *Allantonematiodae*, Para sitylenchidae, *Lotonchiidae*, *Tetradonematidae*, *Sphaerularidae*, *Mermithidae*, *Phaenopsitylenchidae*, *Steinernematidae* and *Heterorhabditidae* have boteutial as bio control agents (Adeolu *et al*., 2016; Ali *et al*., (2010).

12.2 Life Cycle:

Nematode-bacterium complexes called entomopathogenic nematodes exist. The infective juvenile (IJS), which is not feeding and is in development arrest, seeks for insect hosts and starts infections. The namatodes enter the insect body cavity after locating a host, typically via. mouths, anuses, spiracles, and thin cuticle areas are examples of natural bodily openings. Symbiotic bacteria after the nematodes are inside the human cavity (Maurya *et al*., 2020). The nematode's stomach releases (*Xenorhabdus* for *Steinernematida* and *Photorhabdus* for *Heterorhabditids*), which quickly multiplies and kills insects. The nematodes develop into adults after feeding on the bacteria and liquefying host. Juveniles with *steinernematid* infection may mature into males or females. In contrast to *heterorhabditids*, which grow into self-fertilizing hermaphrodites, subsequent genera within a host do generate both men and females. In a matter of days, the life cycle is finished, and hundreds or even thousands of new infectious juveniles emerge in quest of new hosts (Askary *et al*., 2017).

12.3 The Major EPN Species Used as Bio Control:

There are 47 species of EPN that have been classified as bio controlled. The primary EPN employed for biocontrol belongs to the genus Steinernema, of which 38 species are known to parasitize different insect larvae (Griffin 2015).

There are eight other *Heterorhabdities* species that are known, and they are quite parasitic on several lepidopteran and Coleopteran insect larvae (Gassman and Clifton 2017; Gumus *et al*., 2015).

A third genus *Neosteinernema* (was added in 1994) has only one species paraities insect larvae Recently, Rhabditis, (Oscheius) sp. (*Rhabditidae* family) from Andhra Pradesh and Kerala has been reported which found effective against a variety of insect pests., List of Identified species of EPN in as follows:

A. *Steinernema*:

1.	*S. abbasi*	20.	*S. loci*
2.	*S. affine*	21.	*S. masoodi*
3.	*S. anatoliense*	22.	*S. monticolum*
4.	*S. arenarium*	23.	*S. neocurtillae*
5.	*S. asiaticum*	24.	*S. oregonense*
6.	*S. bicornutum*	25.	*S. pakistanense*
7.	*S. carpocapsae*	26.	*S. puertoricense*
8.	*S. caudatum*	27.	*S. rarumf*
9.	*S. ceratophorum*	28.	*S. riobrave*
10.	*S. cubanum*	29.	*S. ritteri*
11.	*S. diaprepesi*	30.	*S. scapterisci*
12.	*S. dutkyi*	31.	*S. scarabaei*
13.	*S. feltiae*	32.	*S. seemae*
14.	*S. glaseri*	33.	*S. siamkayai*
15.	*S. intermedium*	34.	*S. tami*
16.	*S. karii*	35.	*S. thanhi*
17.	*S. kraussei*	36.	*S. thermophilum*
18.	*S. kushidai*	37.	*S. weiseri*
19.	*S. longicaudum*		

B. *Heterorhabditis:*

1.	*H. argentinensis*	5.	*H. indica*
2.	*H. bacteriophora (=H. heliothidis)*	6.	*H. marelatus*
3.	*H. brevicaudis*	7.	*H. megidis*
4.	*H. hawaiiensis*	8.	*H. zealandica*

C. *Neosteinernema:*

a. *N. longicurvicauda*

Pests Attacked: Many of the insect pests that entomopathogenic nematodes are successful against are included in the table below: Important Entomopathogenic nematodes:

S.No.	Commodity	Insect Pests	EPN Species
1.	Artichokes	Artichoke plume	*Steinernema carpocapsae* moth
2.	Berries	Root weevils	*Heterorhabditis bacteriophora*
3.	Citrus	Root weevils	*Steinernema riobravis*
4.	Cranberries	Root weevils	*Heterorhabditis bacteriophora*
5.	Cranberry girdler	Root weevils	*Steinernema carpocapsa* *Steinernema carpocapsae*
6.	Mushrooms	Sciarids	*Steinernema feltiae* *Heterorhabditis bacteriophora*
7.	Or namentals	Root weevils	*Heterorhabditis megidis*
8.	Wood borers	Fungus gnats	*Steinernema carpocapsae*
9.	Fungus gnats	Fungus gnats	*Heterorhabditis bacteriophora*
10.	Turf	Scarabs	*Heterorhabditis bacteriophora*

12.4 Culturing of EPN Nematodes:

In vitro or in vivo methods can be used to generate large quantities of endo-pathogenic nematodes;

A. In-vivo Production of EPN:

EPN can be produced in vivo with a very little initial outlay and in a very straightforward manner. The larvae of wax moths are frequently used to raise nematodes. They can easily be raised in-vivo in the lab on Galleria mellonella because *Steinernematids* and *Heterorhabditis* infect and proliferate in a wide variety of insects. In-vivo manufacturing involves the following steps:

- **Infecting Galleria:** Warming the IJ suspension to room temperature (20–24°C). After that, the nematodes are briefly looked at under a dissecting microscope. While live dauers actively move about, dead dauers are often straight. To produce a suspension with close to 200 nematodes/ml, 1 ml of suspension is diluted in the necessary amount of sterile distilled water (sdw). After counting the IJs, the suspension is changed to 200 nematodes per millilitre. It is equally dispersed to place 1ml of the nematode suspension on a 9.0cm Whatman # 1 filter paper within the lid of a 100 x 14 mm plastic petri dish (Imperiali *et al*., 2017). The following addition is 10 conditioned Galleria larvae. The target number of nematodes per larva is around 20. The bottom of the inverted petri dish is placed over the lid, which contains nematodes and Galleria (Jaffuel *et al*., 2019). The petri dishes are labelled and kept at room temperature in a plastic bag (to keep moisture

in). 5-7 days after infection, the infected larvae are put into white traps. When grasped with forceps, steinernema-infected larvae will be limp and brownish brown. Larvae with heterorhabditis infection turn brick red and become limp (Jaffuel *et al*., 2017).

- **Harvesting:** A 9.0 cm Whatman # 1 filter paper is placed in a large glass petri dish (150 x 20 mm) with the concave side up to create white traps. After that, it undergoes a 20-minute autoclave at 121 C. 70 ml of sdw (0.1% formalin) are added to the petri dish. The watch glass is not submerged in water. The watch glass has the filter paper wrapped around it so that it touches the liquid surface. Ten to thirty infected larvae are placed on the filter paper above the watch glass edge. 10–12 days after infection, IJs will begin to leave the body. Nematodes should be gathered every day starting when they start to appear until production stops (3–4 days).
- **Preparation for storage:** IJs are left to agglomerate in the beaker for rinsing. The supernatant is then removed using aspiration or decantation, and more sdw is added as necessary (2-4 times) to make the suspension clean. Once-rinsing the suspension with 0.1% formalin is an option if it seems extremely polluted. It is possible to accelerate the settling process by centrifuging at 300 rpm for 1 minute. In the end, the nematodes are moved to a storage container (Maurya *et al*., 2018).

B. In-Vitro Production of EPN:

The substrates used to culture Steinernematids and Heterorhabditids in the past include potato mash (McCoy and Glaser, 1936), pulverized veal pulp (McCoy and Girth, 1938), and dog food. Today, a medium based on chicken offal is widespread.

Monoxenicity (*i.e*., the worm and associated bacterium as the only biotic agents), the utilization of primary form bacteria, a sizable surface area on which the nematode may grow, a sterol source for the nematode, and a food source for the bacterium appear to be key elements. The highest surface-to-volume ratio with sufficient interstitial space is offered by polyether polyurethane. Large autoclave bags or glass flasks are used as raising containers (Bhat *et al*., 2019).

- **Preparation of rearing flasks:** During this treatment, rubber gloves are highly recommended. Small (1 cm in diameter) foam pieces with a chicken, duck, or turkey offal homogenate. Researchers suggest a weight ratio of 12.5 parts medium to 1-part foam. When the foam is squeezed, medium should flow out while the pores of the foam should still be plainly visible. Foam homogenate mixture is poured into the flasks until it reaches the 250–300 ml mark (about 100g) (Bhat *et al*., 2020). The flasks' mouths are thoroughly cleaned, closed with cotton fabric wrapped in cheese cloth and autoclaved at 121° C for 20 minutes.
- **Inoculation with bacteria:** Liquid cultures of the relevant bacterial strain's primary form should be incubated the day before the flasks are to be manufactured. One test tube should be used to inoculate one flask with the bacterium. Each test tube should contain 5ml of nutritional broth (Liao *et al*., 2017). To reach room temperature, the autoclaved flasks are allowed to cool. One tube's worth of bacteria is introduced into each flask by pouring it into them. The mixture is then shaken to distribute the broth and bacteria evenly throughout the foam substrate, and it is then kept at 25°C for a couple of days to allow the bacterial population to grow (John *et al*., (2019 a).

- **Inoculation with nematodes:** When monogenic cultures of bacteria are available, the IJs injection is carried out, which will cause the bacteria to begin to grow. You can divide a flask into around seven new ones. It is important to take precautions to keep the transfers monogenic. Within two weeks, the flasks will be prepared for harvest.
- **Harvesting:** On a 20-mesh sieve (20 meshes/inch), the foam may be heaped up to a height of 5 cm. The water level in the pan of tap water is adjusted such that the sieve is submerged just behind the foam. Pouring water over the foam is not advised since it will wash water particles into the foam. 95% of IJs will move into the water in 2 hours. To get rid of debris and inactive IJs, the nematodes may need to be sedimented and rinsed. The IJs may then be allowed to pass through a sieve with a mesh size of 500. Nematodes that have been rinsed from the flask's inside should also be permitted to pass through the 500-mesh sieve to get rid of any debris (John *et al*., (2019 b).

12.4.1 Advantages of *Entomopathognic* Nematodes:

- *Entomopathogenic* nematodes can be employed effectively on a variety of insect pests due to their broad host range.
- The *Xenorhabdus* bacterium produces the enzymes that cause these nematodes to kill their insect hosts in two days.
- On synthetic media, these nematodes can be cultivated. This enables commercial manufacture, increasing their availability as a product. Nematodes that are *entomopathogenic* have long infectious phases. When kept at the right temperature, the nematodes can survive for several months. Generally, keeps for three months at ambient temperature (60° to 80° F) or six months in a refrigerator (37° to 50° F).
- They can also withstand being combined with different fertilizers, herbicides, and insecticides.
- In vertebrates, EPN or their symbiotic bacteria cannot grow. Because of this, using nematodes to control insect pests is both safe and friendly to the environment.

12.4.2 Problems Associated with The Use of *Entomopathogenic* Nematode:

Although they are plainly underutilized, *Entomopathogenic* nematodes are highly adaptable and useful against a wide range of soil and covert insect pests in many farming systems. The limitations of EPN are similar to those of other biological control agents in that they are living things that need particular environments in order to function. In this way, chemical pesticides have less restriction than desiccation or ultraviolet radiation, which quickly inactivate insecticidal nematodes. Likewise, nematodes work well (Pandey *et al*., 2020).

More influenced by substandard soil type, thatch depth, and irrigation frequency, and operating within a smaller temperature range than chemicals (Georgis and Gaugler, 1991). Nematode-based insecticides are incompatible with a number of agricultural chemicals, cannot be kept in spray tanks for extended periods of time, and may become inactive if stored in high temperatures. Some species require different screen widths, and some species cannot be treated with high-pressure application equipment. Additionally, leftover nematodes cannot be sprayed the next year (Pant *et al*., 2020). Although there are issues with chemicals as well (mammal toxicity, resistance, groundwater pollution, etc.), their usage is supported by a substantial body of research.

In order to incorporate nematodes into IPM systems more quickly, users will need to become more proficient with their utilization. Nematodes are naturally occurring and necessary for human consumption, according to the United States Environmental Protection Agency (EPA). Therefore, they are exempt from registration requirements (Sajnaga and Kazimierczak 2020).

Formulation:

The nematodes prepared in several forms of carriers, like clay, vermiculite, and gel-forming polyacrylamides, can be kept for a minimum of three months at ambient temperature and six months under refrigeration. Bait formulations generally consists of a mixture of carrier (eg. wheat bran or peanut hulls etc.), a feeding stimulant (sucrose, glucose and molasses) and toxicant. A number of commercial formulations are available in the USA, Switzerland, Germany and U.K. However, in India single commercial formulations ECOMAX is available. It is prepared from nematodes, *S. carpocapsae and H. bacteriophora* by Good value, Industrial Assurance Bldg. Church gate, Bombay. DD-136 strain of *S. carpocapsae* is also available in several laboratories (Shapiro-Ilan *et al*., 2020).

Compatibility and Application Methods:

Under field conditions, infectious juveniles of EPNs are compatible with most agricultural pesticides. Furthermore, a lot of substances that were known to be harmful only had a transient effect because the nematodes recovered after the exposure (Singh *et al*., 2019). Common agrochemical equipment including as mist blowers, fan sprayers, electrostatic sprayers, small pressurized sprayers, and helicopters can be used to apply nematodes. They can be supplied with any common nozzle type sprayer and withstand pressures up to 300 lb/sq. in. In order to keep the land sufficiently moist, nematodes should be applied and irrigation should resume (Heve *et al*., 2018). Applying should ideally be done in the evening to prevent UV rays from the sun and extreme heat. There are a number of application methods which can be utilized for this purpose like;

- **Spraying**: Spraying of nematode directly on to the soil surface of a dosage of about 100,000 IJs/plant or 2.5-7.5 billion IJs/ha is effective for pest control. Capsule prepared from wheat bran (5% w/w) with calcium alginate which may contain 1000-2000 nematode capsule. These capsules are buried in soil and 70-80 capsule/ plant could be used (Stevens and Lewis 2017).
- **Liquid baits:** Nematodes are mixed with 56% sucrose solution and small droplets containing nematodes can be applied.
- **Punch and syringe:** This method is used in case of forest trees. Above 1 ml. of nematode containing medium is inoculated in hole by syringe.
- **Trap like bands:** Nematodes can be applied to nylon pack cloth bands around, wrapped around tree trunk to control insects. Cardboard bands cantaining *S. carpocapsa* around.
- **Pellet baits:** Wheat bait pellets were prepared from wheat bran-wheat flour (50% each).

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