**Entomopathogenic Nematodes : An overview**

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**Abstract**

Entomopathogenic (or Insect – Pathogenic) nematodes are a group of soil – dwelling roundworms which kill insects that live in, on or near the soil surface, usually closely associated with plants. They are small parasitic roundworms that infects and often quickle kill insects that live underground and also referred as beneficial or insecticidal nematodes. These namatodes are species of the genera *Steinernema*  and *Heterorhabditis* of the phylum Nematoda. EPN are found under diverse ecological conditions including cultivated fields, forest, grasslands, deserts and ocean beaches. The can be best biological control agents for soil dwelling stages of many insect – pests and are fast acting in killing target insect – pests in 24 – 48 hours. EPN are extraordinarily lethal to many important soil insect pests, yet are safe for plants and animals.

Key Words: Nematode, entomopathogenic, insect – pest.

**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.

Based on the feeding habits nematods may be classified into three main groups

1. **Saprophagous**: living, on dead organic matter of plant and animal origin and micro – organisms associated with decay,
2. **Predators**: Feeding on small animals including namatodes.
3. **Parasites**: infesting 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. Entomopathogenic nematodes fit nicely into integrated pest management programs because they are considered non toxic to humans, relatively specific to their target pests and can be applied with standard pesticide equipment (Shapiro – Ilan et. al. 2006).

Entomopathogenic namatodes have been exempted from the U.S. environmental protection agency (EPA) pesticide registration . There is no need for personal protective equipment and re – entry restriction. Insects resistance problems are unlikely. most bio-control agents require days or weeks to kill insects pest, yet nematodes, working with their symbiotic bacteria, kill insect – pest, in one or two days. Dozens of various insect – pests are susceptible to infection & yet no adverse effects have been shown against non – targets n filed condition (Georgis et. al., 1991). Nematodes do not require specialzed application equipment as they are compatible with standard agro – chemical equipment including pressurized mist, electrostatic fan and aerial sprayers. Nematodes belong to the families Allantonematiodae, Para sitylenchidae, Lotonchiidae, Tetradonematidae, Sphaerularidae, Mermithidae, Phaenopsitylenchidae, Steinernematidae and Heterorhabditidae have boteutial as bio control agents.

**Life Cycle :-**

Entomopathogenic nematodes are a nematode – bacterium complex. The non feeding and developmentally arrested infective juvenile (IJS) seeks out insect hosts and initiates infections. When a host been located, the namatodes penetrates penetrate into the insect body cavity, usually via. natural body openings suh as mouth, anus, spiracles or area of thin cuticle. One the nematodes are in the body cavity, a symbiotic bacterium.(*Xenorhabdus* for *Steinernematida* and *Photorhabdus* for *Heterorhabditids*) is released from the nematode gut, which multiplies rapidly and causes rapid insect death. The namatodes feed upon the bacteria and liquefying host and mature into adults. Steinernematid infective juveniles may become males or females. Where as *Heterorhabditids* develop into self – fertilizing hermaphrodites, although subsequeut generatins within a host produce males and females as well. The life cycle is completed in a few days, and hundreds or thousands of new infetive juveniles emerge in search of fresh hosts.

**The Major EPN species used as Bio control:**

Forty seven species of EPN have been reported as bio contrl. The major EPN used for bio-control fall under genus – *Steinernema* in which Thirty Eight species are reported to parasite on various insect larvae. Another eight species from genus *Heterorhabdities* are reported which are highly parasitic on some lepidopteran and Coleopteran insect larva. 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:

*1.* ***Steinernema :***

*20. S. loci*

*21. S. masoodi*

*22. S. monticolum*

*23. S. neocurtillae*

*24. S. oregonense*

*25. S. pakistanense*

*26. S. puertoricense*

*27. S. rarum f*

*28. S. riobrave*

*29. S. ritteri*

*30. S. sangi*

*31. S. scapterisci*

*32. S. scarabaei*

*33. S. seemae*

*34. S. siamkayai*

*35. S. tami*

*36. S. thanhi*

*37. S. thermophilum*

*38. S. weiseri*

*1. S. abbasi*

*2. S. affine*

*3. S. anatoliense*

*4. S. arenarium*

*5. S. asiaticum*

*6. S. bicornutum*

*7. S. carpocapsae*

*8. S. caudatum*

*9. S. ceratophorum*

*10. S. cubanum*

*11. S. diaprepesi*

*12. S. dutkyi*

*13. S. feltiae*

*14. S. glaseri*

*15. S. intermedium*

*16. S. karii*

*17. S. kraussei*

*18. S. kushidai*

*19. S. longicaudum*

**2. *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*

1. ***Neosteinernema***
2. *N. longicurvicauda*

**Pests Attacked:**

Entomopathogenic nematodes are effective against a large number of insect pests, many of which are listed in t, below : Important Entomopathogenic nematodes :

**Commodity Insect Pests EPN Species**

Artichokes Artichoke plume *Steinernema carpocapsae*

moth

Berries Root weevils *Heterorhabditis bacteriophora*

Citrus Root weevils *Steinernema riobravis*

Cranberries Root weevils *Heterorhabditis bacteriophora*

 Cranberry girdler *Steinernema carpocapsa*  *Steinernema carpocapsae*

**Mushrooms**  Sciarids *Steinernema feltiae*

 *Heterorhabditis bacteriophora*

Or namentals Root weevils *Heterorhabditis megidis*

Wood borers *Steinernema carpocapsae*

 *Heterorhabditis bacteriophora*

Fungus gnats *Steinernema feltiae*

Turf Scarabs *Heterorhabditis bacteriophora*

Mole crickets *Steinernema riobravis,*

*Steinernema scapterisci*

Billbugs *Heterorhabditis bacteriophora*

Armyworm, *Steinernema carpocapsae*

*Steinernema carpocapsae*

 Cutworm, Webworm

**Culturing of EPN nematodes:**

Entomopathogenic nematodes can be mass produced by in vitro or in vivo mthods;

**(a) In-vivo Production of EPN**

The in vivo production process of EPN is very simple and requires only minimal initial investment. The wax moth larvae are commonly used to rear nematodes. Since *Steinernematids* and *Heterorhabditis* infect and reproduce in a broad spectrum of insects, they can be readily reared in-vivo in the laboratory on *Galleria mellonella*. The steps of in-vivo production are as follows:

Infecting *Galleria:* The IJ suspension is warmed to room temperature (20-24°C). The nematodes are then examined briefly under dissecting microscope. Dead dauers are generally straight while the live dauers actively move about. 1 ml suspension is diluted in an appropriate quantity of sterile distilled water (sdw) to yield a suspension near 200 nematodes/ml. The IJs are counted and the suspension is adjusted to 200 nematodes/ml. 1ml of the nematode suspension on a 9.0cm Whatman # 1 filter paper in the lid of a 100 x 14 mm plastic petri-dish is evenly distributed. Then 10 conditioned *Galleria* larvae are added. The objective is to have about 20 nematodes per larva. The lid (containing nematodes and *Galleria)* is covered with the inverted petri-dish bottom. The petri-dishes are labeled and stored in a plastic bag (to conserve moisture) at room temperature. The infected larvae are placed into white traps (White, 1927) 5-7 days after infection. *Steinernema-*infected larvae will be yellowish brown and limp when held with forceps. *Heterorhabditis*-infected larvae turn brick red and are also limp.

**Harvesting**: To make white traps (white, 1927), a 9.0 cm Whatman # 1 filter paper is placed in a concave -side- up watch glass in a large glass petri dish (150 x 20 mm). It is then autoclaved for 20 minutes at 121° C. About 70 ml sdw or 0.1 % formalin is poured into the petri-dish. No water is put into the watch glass. The filter paper is wrapped over the watch glass so that it comes into contact with liquid surface. The infected larvae are placed (10-30 as they fit) on the filter paper over the edge of the watch glass. IJs will start to exit 10-12 days after infection. Once nematodes begin to appear, they should be harvested daily until production drops (3-4 days).

**Preparation for storage** : To rinse IJs, they are allowed to settle in the beaker. Then the supernatant are aspirated or decanted and more sdw is added until the suspension is clean (2-4 times). If the suspension appears particularly contaminated, it may be rinsed once with 0.1 % formalin. Centrifugation at 300 rpm for 1 minute may be used to speed the settling process. Finally, the nematodes are, transferred to a storage container.

 **(b) In-Vitro Production of EPN:**

In the past, *Steinernematids* and *Heterorhabditids* have been cultured on a variety of substrates i.e. Potato mash (McCoy and Glaser, 1936), ground veal pulp (Mc Coy and Girth, 1938) and dog food (House et., al., 1965, Hara et, al., 1991). Currently, a medium based on chicken offal (Bedding, 1984) is common. The important factors seem to be monoxenicity (i.e., the nematode and associated bacterium as the only biotic agents), the use of primary form bacteria, a large surface area on which the nematode may grow, a sterol source for the nematode and a food base for the bacterium. Bedding (1981, 1984) has developed a technique whereby huge numbers of nematodes may be economically produced using a chicken offal medium on a porous foam substrate. Polyether polyurethane provides the largest surface-to-volume ratio while providing adequate interstitial space (Bedding, 1986). Glass flasks or large autoclavable bags serve as rearing containers.

**Preparation of Rearing flasks**: Wearing of rubber gloves during this procedure is highly desirable. Impregnate small foam pieces (1 cm diameter) with chicken, duck or turkey offal homogenate. Bedding (1984) recommends 12.5 parts medium to I part foam, by weight. The pores of the foam should still be clearly visible, but medium, should ooze out when the foam is squeezed. The flasks are filled with foam homogenate mixture to the 250-300 ml mark (about 100g). The mouth of the flasks is wiped well and plugged with cotton wrapped in cheese- cloth, and then autoclaved for 20 minutes at 121° C.

**Inoculation with bacteria:** The day before the flasks are to be prepared, liquid cultures of the primary form of the appropriate bacterial strain should be incubated. Cells of the bacterium should be asceptically transferred to 5ml of nutrient broth in a test tube (one tube per flask to be inoculated). The autoclaved flasks are allowed to cool to room temperature. By pouring the contents of one tube of bacteria is inoculated into each flask. It is then shaked to mix the broth and bacteria throughout the foam substrate and stored for 2-3 days at 25°C to allow the bacterial population to build up.

**Inoculation with nematodes**: When monoxenic cultures of bacteria are available, the inoculation with IJs is done which will start to multiply on the bacteria. One flask can be divided into about seven new ones. Care should be taken to maintain monoxenity during the transfers. The flasks will be ready to harvest in about 2 weeks.

**Harvesting:** The foam may be piled 5cm deep on a 20 mesh sieve (20 meshes /inch). The sieve is placed in a pan of tap water with water level adjusted so that the foam is just submerged. The water over the foam should not be poured as this washes particles of homogenate the water. Within 2 hours 95 % of the IJs will migrate into the water (Bedding, 1984). The nematodes may be sedimented and rinsed if necessary to remove particulate matter and inactive IJs. The IJs may then be permitted to migrate through a 500- mesh sieve. Nematodes rinsed from the inside of the flask should also be allowed to migrate through the 500-mesh sieve to remove particulate matter.

**ADVANTAGES OF ENTOMOPATHOGNIC NEMATODES:**

* The Entomopathogenic nematodes have a very wide host range that they can be used successfully on numerous insect pests.
* These nematodes kill their insect hosts within 2 days due to enzymes produced by the *Xenorhabdus* bacteria.
* These nematodes can be grown on artificial media. This allows for commercial production which makes them a more available product
* The infective stages of Entomopathogenic nematodes are durable. The nematodes can stay viable for months when stored at the proper temperature. Usually three months at a .room temperature of 60° to 80° F and six months when refrigerated at 37° to 50° F.
* They can also tolerate being mixed with various insecticides, herbicides and fertilizers.
* EPN or their symbiotic bacteria can not develop in vertebrates. This makes nematode use for insect pest control safe and environmentally friendly.

**PROBLEMS ASSOCIATED WITH THE USE OF ENTOMOPATHOGENICNEMATODE**

Entomopathogenic nematodes are remarkably versatile in being useful against many soil and cryptic insect pests in diverse cropping systems, yet are clearly underutilized. Like other biological control agents, EPN are constrained by being living organisms that require specific conditions to be effective. Thus, desiccation or ultraviolet light rapidly inactivates insecticidal nematodes while chemical insecticides are less constrained. Similarly, nematodes are effective

Within a narrower temperature range than chemicals, and are more impacted by suboptimal soil type, thatch depth, and irrigation frequency (Georgis and Gaugler, 1991). Nematode-based insecticide may be inactive if stored in hot conditions, cannot be left in spray tanks for long periods, and are, incompatible with several agricultural chemicals. Certain species cannot be applied with high-pressure application equipment; unused nematodes cannot be applied in the following year; different species require different screen sizes Chemicals also have problems (e.g., mammalian toxicity, resistance groundwater pollution, etc.) but a large knowledge base has been developed to support their use. Accelerated implementation of nematodes into IPM systems will require users to be more knowledgeable about how to use them effectively. The United States Environmental Protection Agency (EPA) has ruled that nematodes are require exempt from registration because they occur naturally and require by man.

**Formulation**: The nematodes formulated in various carriers such as clay, vermiculite and gel forming polyacrylamides can be stored for at least 6 months under refrigeration and 3 months at room temperature. 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.

**Compatibility and application methods:** Infective juveniles of EPNs are compatible with most of the agricultural chemicals under field conditions. Moreover many chemicals noted to be toxic had only temporary effect only, as the nematodes recovered when the exposure ended. Nematodes can be applied with common agrochemical equipment including small pressurized sprayers, mist blowers, electrostatic sprayers fan sprayers and Helicopters. They stand pressure of 300 lb/sq. in. and can be delivered with all common nozzle type sprayers. The nematode should be applied to irrigated field and there should be again irrigation to maintain sufficient moisture. Application should be done preferably during evening to avoid UV radiation from sun and high temperature. 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 hematode capsule. These capsules are buried in soil and 70-80 capsule/ plant could be used.

**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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