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Mass Production of Entomopathogenic Neamatodes

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INTRODUCTION

Nematodes are simply roundworms the most numerous multicellular animals on earth constitute the phylum *Nematoda.* Nematodes are one of the most abundant groups of living animals, and although morphologically they are very simple, they have exploited a wide range of diverse habitats including invertebrates. Nematodes are devoid of segmentation and appendages and may be free-living, predaceous or parasitic. A handful of soil contains thousands of the microscopic worms, many of them parasites of insects, plants or animals. Free-living species are abundant, including nematodes that feed on bacteria, fungi, and other nematodes, yet the vast majority of species encountered are poorly understood biologically. There are nearly 20,000 described species classified in the phylum Nematoda. The nematodes possess digestive, nervous, excretory, and reproductive systems, but lack a discrete circulatory or respiratory system. In size they range from microscopic (0.3 mm) to conspicuous (over 8 meters).

Many of the parasitic species cause important diseases of plants, animals, and humans. Other species are beneficial in attacking insect pests, mostly sterilizing or otherwise debilitating their hosts. The only insect-parasitic nematodes possessing an optimal balance of biological control attributes are Entomopathogenic (EPNs) or insecticidal nematodes in the genera *Steinernema* and *Heterorhabditis*.

The EPNs are microscopic cylindrical worms and devoid of segmentation. The Department of Environment and Rural and Marine Environments classify them as Biological Control Organisms, with the same regulation as the Auxiliary Insects, and not as micro-organisms. . The insect parasitic (entomopathogenic) nematode, *Heterorhabditis bacteriophora*, was first described in 1975 as a new genus, species, and family (Heterorhabditidae) of Rhabditida (Poinar, 1975).

Hundreds of researchers representing more than fourty countries are working to develop nematodes as biological insecticides. Nematodes have been marketed on every continent except Antarctica for control of insect pests in high-value horticulture, agriculture, and home and garden niche markets.

Research on EPN in India and in Abroad:

The two prominent genera of Entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis* having interaction with insects and are considered globally as highly pathogenic to insects and are being used in integrated pest management. Management of insects through EPNs during last two decades is increased to a surprising level in developed countries and now EPN based several biopesticides are widely marketed in Europe and America as well as considered as second most adopted biopesticide after *Bacillus thuringiensis*.

In India, work on nematode parasites of insect was started by Basir in 1940 and he did a lot during 1940-1970. Indigenous isolates of EPNs have been isolated from different Indian states and Union Territories. Among the indigenous isolates, five have been described as new species *viz*. *H. indica* from Tamil Nadu, *S. thermophilum* from New Delhi, *S. masoodi* and *S. seemae* from Kanpur and *S. meghalayensis*. Since then a number of isolates of both Steinernema and Heterorhabiditis species have been intercepted and reared in several laboratories.

Status of EPNs in Abroad Biology of EPN and biological control of insect pests using steinernematid and heterorhabditid nematodes has been studied by few workers. Up to now about 111 species of EPN are known worldwide belonging to 3 genera, namely Steinernema, Heterrhabditis and Neosteinernema and also studied the insecticidal properties of EPNs.

Mode of action of EPN:

When a host has been located, the nematodes penetrate into the insect body cavity, usually via natural body openings (mouth, anus, spiracles) or areas of thin cuticle. The Infective Juveniles (IJs) also enter the haemocoel by penetrating the exoskeleton using a 'buccal tooth'-like structure. Once in the body cavity, a symbiotic bacterium i.e. *Xenorhabdus* associated with *Steinernematids* and *Photorhabdus* associated with Heterorhabditids is released from the respective nematode gut, which multiplies rapidly and causes rapid insect death normally in 24 - 48 hrs. The nematodes feed upon the bacteria and liquefying host, and mature into adults. The nematodes start developing, feed on the bacteria and host tissues metabolized by the bacteria and go through 1-3 generations. Depleting food resources in the host cadaver leads to the development of a new generation of IJs that emerges from the host cadaver in search of a new host.

Mass production of EPN:

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) kill insects with the aid of mutualistic bacteria. The nematode-bacteria complex is mass produced for use as biopesticides using in vivo or in vitro methods, i.e., solid or liquid fermentation. In vivo production (culture in live insect hosts) is low technology, has low startup costs, and resulting nematode quality is high, yet cost efficiency is low. In vitro solid culture, i.e., growing the nematodes and bacteria on crumbled polyurethane foam, offers an intermediate level of technology and costs.. In vitro liquid culture is the most cost-efficient production method but requires the largest startup capital and nematode quality may be reduced. Liquid culture may be improved through progress in media development, nematode recovery, and bioreactor design.

In-vivo method:

- The basic technique was first describe by Dutky *et al*. in (1964)
- Insect host serve as small biological reactor for mass production of EPN
- The larvae of wax moth, *Galleria mellonella* are commonly used as a factitious host in *in-vivo* method of production (several lepidopterous larvae also been used for mass production of *steinernema* and *Heterorhabditis*)
- The most common insect host used for *in -vivo* production is the last instars of the wax moth (*Galleria melonella*), because of its high susceptibility to most nematodes.
- Using the *in-vivo* process, yields between $0.5x10^5$ - $4x10^5$ infective juveniles per larva, epending on the nematode species, have been obtained.
- During the past few years a distinct cottage industry has emerged in the USA which utilizes the *in-vivo* process for nematode mass-production for sale, especially in the home lawn and garden markets.

- The yield of infective juveniles varies with nematode species, insect host species, environmental condition and nutrient status of host.

CONCLUSION

EPN is a proved biopesticides consist as an excellent alternate tool for integrated pest management and there is scope for biocontrol workers to test its efficacy against a wide variety of major insect pests. It has a potential to control of several insect pests and there are number of insect pest which are successfully controlled in field as well as in laboratory condition. There is further need to obtain basic information on biology, behaviour, ecology and genetics of these nematodes and their associate bacterium.

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