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A Review on the control of few endemic pests by Entomopathogenic nematodes for sustain production of Crops biologically

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ABSTRACT
Insect pests are responsible to cause a huge crop loss annually. Chemical control measures are the first sought options among the growers but these chemicals or pesticides have negative effects on soil fertility, soil health, environment and human health. Nematode is a tiny worm like free-living organism. Being abundant in nature, it can be potentially utilized for the pest management. Nematodes are known to parasitize and kill the insects. Such type of nematodes are referred to as Entomopathogenic nematode (EPNs). EPNs carry specific bacteria in their guts which helps them to kill their target host. Thus they form nematode-bacteria complexes for which they are considered as a potential agents for the control of insect pest. Entomopathogenic nematode is ubiquitous in the world and they are mainly soil-inhabitant. EPNs consist of two major families, Stenermatidae and Hetrorhabditidae. About 90 species of Stenernematidae and 20 species Heterorhabditidae have been described according to Labaude, S and Griffin, T (2018). EPNs carry specific bacteria in their guts which helps them to kill their target host. Thus they form nematode-bacteria complexes for which they are considered as a potential agents for the control of insect pest. We have a great scope in controlling insects biologically through entomopathogenic nematodes to have a sustainable crop production. In this paper, a review is made on the prospects of utilization of entomopathogenic nematodes in pest management worldwide and in India.
Keywords: *Biocontrol agent, Entomopathogenic nematode, Pest*

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INTRODUCTION

Nematode is a tiny free-living organism which is found in almost every niche of our environment like in marine, freshwater, soil ecosystems and in plant and animal as parasite. Nematode is an abundant organism which is omnipresent in our environment and their population is just next to insect population. Due to their abundance in nature, it can be potentially utilize for an insect pest management. In India, it is reported that more than 400 species in plant and soil nematodes are still known from type localities only [1]. Dhaliwal *et al.* [4] have reported that about 10,000 of insect pest are known to cause damage to the crop. Nematodes are known to parasitize and kill the insects. Such type of nematodes is referred to as Entomopathogenic nematode (EPNs). EPNs carry specific bacteria in their guts which helps them to kill their target host. Thus they form nematode-bacteria complexes for which they are considered as a potential agent for the control of insect pest. Entomopathogenic nematode is ubiquitous in the world and they are mainly soil-inhabitant. EPNs consist of two major families, Stenermatidae and Hetrorhabditidae. About 90 species of Stenernematidae and 20 species Heterorhabditidae have been described according to Labaude, S. and Griffin, T. [12]. Poinar [14] has first described the insect parasitic (entomopathogenic) nematode, *Heterorhabditis bacteriophora*, as a new genus, species in 1975. Steiner has described the first EPNs name as *Aplectana kraussei* (now *Steinernema kraussei*) in 1923 followed by *Neoplectana glaseri*.

Both *Heterorhabditis spp.* and *Steinernema spp.* during the non-feeding, third stage juvenile is found to be in symbiotic association with bacteria of the genus *Photorhabdus luminescens for H. Bacteriophora and Xenorrhabdus for Steinernema carpocapsae.* This symbiotic bacterium (Enterobactericeae) lives in its intestine. The infected 3rd stage juveniles survive outside the insect and search for hosts and wait for host to pass by [13]. The only free living nematode stage is the developmentally arrested third stage duaer juvenile (DJ). Generally DJs are non-pathogenic. They enter the haemocoel of an insect host within a few days. On depletion of nutrients, the nematodes produce next generation juveniles which leave the host cadaver seeking new hosts [8]. Kary *et al.* [10] found that *Heterorhabditis bacteriophora* is a good biocontrol against cotton boll worm (*Helicoverpa armigera*).

IMPORTANCE OF EPNs

Entomopathogenic nematodes can be used as an integrated pest management or IPM programs due to their non-toxic nature to environment and relatively specific to their target pest(s) and can be applied with standard pesticide equipment [11].EPNs treatment with agrochemicals is a cost-effective way. Entomopathogenic nematodes species like Allantonematidae, Diplogasteridae, Heterorhabditidae, Mermithidae, Neotylenchidae, Rhabditidae, Sphaerulariidae, Steinermnematidae and Tetradonematidae that attack insects and kill or sterilize them, or alter their development are used as an excellent biological control agents which are infective to some these order like Coleoptera, Lepidoptera, Hemiptera. This nematode has paves the way in developing successful bio-control management of pest. Some nematodes that are commercially available *Steinernema carpocapsae*,*S.feltiae*, S.*riobrave* ,*Heterorhabditis bacteriophora*,*H. marelatus*, and *H. megidis* . Kary *et al.* [10] found that *Heterorhabditis bacteriophora* is a good biocontrol against cotton boll worm (*Helicoverpa armigera*).

Buitenhuis and Shipp [3] investigated that in an ornamental greenhouse where western flower thrips, *Frankliniella occidentalis*, found in a crop can be controlled by using nematode *S. feltiae*.

Some known appropriate pathogen-host targets are: *S. glaseri* against the Japanese beetle; *S. scapterisci* against mole crickets; *S. riobrave* against cutworms and other noctuid larvae, pupae and citrus weevils; and *S. feltiae* against sawfly larvae and fungus gnat larvae.

Factors affecting EPNs

The effects of environmental factors (such as temperature, moisture, aeration, and soil type [esp. texture and chemistry] and biotic factors (species of EPN, targeted insect, age of insect, soil fauna) have been documented by numerous researchers.[5,12,20,7,6].

Protection from UV radiation during application (should be applied early in morning or in the evening). Soil texture has an effect on infective juvenile survival with survival being lowest in clay soils. The poor survival rate in clay soils is probably due to lower oxygen levels in the smaller soil pores. Oxygen may also become a limiting factor in water-saturated soils and soils with contents of organic matter [20, 21].

Sea water has no negative effects on survival of several Heterorhabditis species/ strains [8].

Most failures in efficacy of field applications are related to a poor match between the nematode species and target insect pest. Species of nematodes vary in their host range and in their host-finding behavior. Some nematodes, for example, *Steinernema glaseri* and *Heterorhabditis bacteriophora*, are very active in the soil and search a relatively large area for a host insect, whereas the widely available nematode, *S. carpocapsae*, is relatively sedentary and tend to sit and wait for a host insect to pass by in close proximity. *S. carpocapsae* is classified as an ambusher and is most suitable for mobile pests, for example armyworms and cutworms. *S. riobrave* moves well through the soil. It was originally found in the Rio Grande Valley of Texas, is adapted to warm soils and is more tolerant to tilled soils than are some other nematode species.

Attributes of EPNs as bio-control agent

EPNs has a some good attribute as the potential biocontrol agent such as quick host kill, environment friendly i.e. safe to non-target host, high virulence, broad host range, good searching capacity.

ADVANTAGE AND DISADVANTAGE OF EPNS [16-19]

Advantages: Broad host range, kill the host rapidly within 24-48 hrs after inoculation, ambush nature toward host and safe to the environment.

Disadvantages: Adequate moisture and temperature is required, Limited shelf-life, refrigerated storage required, sensitive to UV radiation**,** show harmful effect on contact of pesticides (nematicides, fumigants and others) as well as change in the soil chemical properties (high salinity, high or low pH, etc.). However it is time consuming and effect laboratory is required for their mass production. Therefore, cost of production is higher as compared to any pesticide.

Rearing methods EPNs

David *et al.*, 2014 has described the three different methods of producing entomopathogenic nematodes through mass production viz., *in vivo* production, in vitro culture in solid media, and in vitro culture in liquid media.

***In vivo* production**

This is the most commonly used method where entomopathogenic nematodes are mass multiplied in live insect host larvae. This method is simple and requires low technology. Under this method , some of the commonly found insect larvae of the greater wax moth, *Galleria mellonella*, the rice moth, *Corcyra cephalonica*, or the mealworm, *Tenebrio molitor*, which are reared in the laboratory [1]. Collection of nematode is done by using White trap method, in which an whatsman filter paper placed on above of the inverted watch glass keeping inside the water filled petridish. The cadaver is placed on the watch glass centre where the migrates away from the host-cadaver. The approach is based on two-dimensional systems that rely on nematode production in trays and shelves [14, 16-17]; Shapiro-Ilan *et al.* [19] stated that this method is not cost effective for scaled-up productions and may be only ideal for small markets or laboratory studies.

***In vitro* production**

This production technique requires sophisticated equipments and media culture. Entomopathogenic nematodes are introduced to a pure culture of their symbiont in a nutritive medium. For commercial mass production large fermenters used to produce huge quantities of entomopathogenic nematodes.

Commercial formulaion

The formulation of entomopathogenic nematodes is prepared as suspensions in liquid, on polyurethane sponge, in gels, or as semidry granules and baits. Storage periods of entomopathogenic nematodes formulation range from 2 to 5 months depending on the nematode species and storage media and conditions. Unlike other microbial control agents (fungi, bacteria and virus) entomopathogenic nematodes do not have a fully dormant resting stage and they will use their limited energy during storage. Grewal *et al.* 2005 reported that 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.

Application of EPNs

EPNs are applied in number of ways. Their application methods includes- spraying, trickle irrigation, liquid baits, pellets baits, as capsule, nylon pack cloth bands, punch and syringe method etc. Spraying of EPN directly on to the soil surface and foliage is the most common method of EPN application. In trickle irrigation method, EPNs are applied to the side of the plant through some button type trickle irrigation outlets. Desiccated nematodes (*Steinernema feltiae*) are mixed with 56% sucrose solution to be applied as liquid baits [6]. Capsules are prepared from wheat bran with calcium alginate containing 1000-2000 nematodes/capsule. These capsules are buried in the soil. About 70-80 such capsules can be used per plant. Nematodes can be applied to nylon pack cloth bands lined with fleece or terrycloth that is wrapped around tree trunks to control gypsy moth larvae, *Lymontria dispar* [11]. *S. carpocapsae* incorporated into cardboard band placed around the trunk of apple trees as an artificial bark substrate, infected 23-73% of codling moth pre-pupae that moved under the barks. Punch and syringe method is used in forest trees, with the help of hammer inoculation hole is made and delivery of about 1 ml of nematode containing medium (aerated 12% gelatin with 4000 nematodes/ml) is done by syringe.

Table-1: Application methods of EPN against some specific pest

Application method	**Pest**
Overhead sprinkler system	Artichoke plum moth
Injection into larval galleries	*Zezeura pyrina*
With liquid fertilizer	*Diabrotica undecemlineata*
Spinning dic sprayers	*Plutella xylostella*
Spraying and irrigation	*Temnorhinus mendicus, Pectinophora gossypiella*
Knapsack sprayer	*Conorhynchus mendicus*
Spraying in dung tubs	Fly maggot
Calcium alginate capsules	*Musca domestica*
Sprayed on soil	*Helicoverpa zea*
Bark sprays with backpack sprayer	*Podosesia aureocincta*

HANDLING AND EFFECTIVENESS

Unsatisfactory results of entomopathogenic nematodes as pest control agents are caused by improper handling, transport, and storage (8). Entomopathogenic nematodes are living organisms, and both biotic and abiotic factors can be detrimental during their applications. Entomopathogenic nematodes work best in sandy soil with a pH between 4 and 8. They are susceptible to freezing, hot temperatures, desiccation, and UV light. More heat tolerant species include *Steinernema ribrave*, *S. glaseri*, and *Heterorhabditis indica*, while *S. feltiae*, *Heterorhabditis megidis* and *H. marelatus* are adapted to cooler temperatures [7]. The nematode efficacy can be enhanced by matching the most appropriate species to the target pests, using the correct rate of a viable nematode product, keeping the treated area wet for at least 8 hours post application and applying during early morning or evening hours to minimize UV exposure and drying conditions. It is also important to inspect entomopathogenic nematodes after receiving them and prior to application to ensure that they are viable (sinusoidal movement of healthy juvenile stages can be observed with a 20× hand lens or microscope).

CONCLUSION

Entomopathogenic nematodes are coming out to be one of the most effective biological control agents. Isolation of various species of EPN has resulted in increasing their population in various localities. It has been found that EPNs are very effective in growing under protected cultivation in Korea and Brazil. High dose of pesticide deteriorate the soil health as well as the other flora and fauna present in the soil ecosystem. Biocontrol agents are mostly confined to the laboratory experiments only; they are yet to be popularized among the farmers. Therefore, in this review paper an attempt has been made present to evaluate the EPNs against endemic insect pest of various crops so that one can develop and utilize an EPN formulation to sustain production of diseased free crops.

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