A Study of Entomopathogenic Nematodes and Their Role in Microbial Control of Pests
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Abstract
An essential part of managing insect pests is the use of entomopathogenic nematodes and in preventing environmental contamination. Their use has been increasing in recent years. So far, about 30 to 40 nematode families are in contact with insects and other vertebrates. Among these families, the group widely studied as the so-called "entomopathogenic nematodes," also known as EPN, are Heterorhabditidae and Steinernematidae. Two species of Oscheius (Oscheius chongmingensis and Oscheius carolinensis) have been added in recent years to the EPNs group, and we expect that several species will be added to EPNs. ENP has a wide range of host insects found in a species of EPN that can attack over 250 different kinds of insects from several families. Suitable environments for EPNs include insect hemocoels, soil pores, or river bottoms that grow in contact with these environments. Occurrence, mobility, distribution, and stability of EPN under the influence of several factors, including intrinsic factors such as behavioral, physiological, and genetic characteristics. Biological nature included are hosted and non-host arthropods, predators, parasites, diseases, and aberrant environmental elements like temperature, moisture content, texture, pH, and UV radiation. Proper mass production and application are essential for the biological control effectiveness of entomopathogenic nematodes (EPN). In addition, there is no problem in applying EPNs because they are simple to spray with common equipment and are compatible with almost all chemical fertilizers, but the compatibility is different from chemical pesticides.

Keywords: Entomopathogenic nematodes, Host range, Commercial assessment

Introduction
Entomopathogenic nematodes (EPNs) are naturally occurring roundworms that inhabit the Enterobacteriaceae family of highly specialized bacteria in a mutually beneficial relationship and have the ability to kill an insect host in 48 hours (Ali et al., 2010; Ramirez et al., 2009). Between the invertebrate parasites, so far, there are about 30 to 40 nematode families in contact with insects and other vertebrates (Koppenhofer et al., 2000; Liu et al., 2000; Shapiro-Ilan & Gaugler, 2002; Somasekhar et al., 2002). From the mentioned families, only the following families Steinernematidae, Rhabditidae, Mermithidae, Neotylenchidae, Allantonematidae, Sphaerularidae, and Heterorhabditidae possess the capacity to be regarded as agents of biological control (Dillman & Sternberg, 2012; Grewal et al., 2001). Among these families, the group that has been widely studied as the so-called "entomopathogenic nematodes," also known as EPN, are Heterorhabditidae and Steinernematidae (Gaugler, 1988; Grant & Villani, 2003; Lortkipanidze et al., 2016; Triggiani & Cravedi, 2011; Weischer, 2000). Two species of Oscheius (Oscheius chongmingensis and Oscheius carolinensis) have been added in recent years to the EPNs group, and we expect that several species will be added to EPNs. In order to control insect infestations, the EPNs are widely used as bio-insecticides to prevent environmental pollution (Ali et al., 2010; Duchaud et al., 2003; Kaya & Koppenhöfer, 1996; Ramirez et al., 2009). As bio insecticides, because they are contagious and free-living, the third stage infective juveniles (IJs) are used able to move in the soil and can hunt the host insects in the soil environment (Degenhardt et al., 2009; Demarta et al., 2014; Strong et al., 1999). At this stage, nematodes are not fed, do not develop, and can survive for many months (Batalla-Carrera et al., 2010; Bathon, 1996; Grewal et al., 2003; Shapiro-Ilan et al., 2013). Insects have known about entomopathogenic nematodes since the 17th century (Nickel, 1984), but the use of nematodes to manage insect pests did not receive much attention until the 1930s (Dillman & Sternberg, 2012; Kaya & Patricia Stock, 1997; Smits, 1996). Before 1929, entomopathogenic nematode was not approved as an insect control agent, and Japanese beetles, Popillia japonica (Newman), were discovered in large numbers on the Tavistock Golf Course near Haddonfield, New Jersey, in 1929 by Glaser and Fox (1930), infected with a nematode (Chandler et al. 1997; Gaugler et al. 1997; Kung et al. 1990; Rasmann & Turlings, 2008; Zhang et al. 2008, Ye et al. 2010). Glaser and his colleagues separated the nematodes from the insect’s cadavers, multiplied them for field experiments, and utilized them to control beetles in Japan in 1931. Following that time, a great deal of work has been done to isolate EPNs from various...
geographic locations so that they might be utilized to control insect pests biologically (Duchaud et al., 2003; Georgis et al., 2006; Ramon Georgis & Kaya, 1998; Hiltpold et al. 2015; Shapiro-Ilan et al. 2013; Vashisth et al. 2013). Therefore, this article reviews the studies of nematodes related to insects and their function in controlling pests, the appropriate way of applying them in the environment to control pests, and the immunity of entomopathogenic nematodes to humans’ bodies and environments.

**Host range and invasion of EPNs**

Because nematodes can kill their hosts very rapidly, there is no chance of a connection between the parasite and its host. Because of their ability to eradicate a wide variety of hosts belonging to various species, EPNs are regarded as having a broad host range (Hallem et al., 2011; Peters, 1996; Strong et al., 1996). It can be concluded from the experiments that more than 250 distinct insect species from more than 75 families in 11 orders could be killed by *Steinernema carpocapsae* Weiser 1955. Nevertheless, varying outcomes have been noted in field investigations because of restricted nematode contact and interaction, as well as because of UV radiation, dryness stress, and temperature variations, all of which aid in lowering EPN infection (Glazer, 1997; Půža et al., 2010; Simões & Rosa, 1996). In order to successfully contact the host, IJs can find a sensitive point in the host for potential pressure, then get, penetrate, and settle in the host body cavity (Sumit Vashisth, 2018). There was no IJ penetration into the body of insects for up to 6 hours in each nematode species/isolated. This is because nematodes need some time to locate a potential host (Lu et al. 2017; Peña et al. 2015).

The chemical signals in the intestinal fluid are important for the host to find nematodes because they can remain as feces and saliva at the host's feeding site. The chemical signals in the gut fluid are very important for nematodes to find the host because they can remain as feces and saliva at the host's feeding site (Kutz et al., 2013). Many researchers conclude that J2 (*Heterorhabditis heliothidis* Khan, Brooks, and Hirschmann) penetrates through the mouth, spiracles, and anus within 2 hours of exposure to host insects. This differential in speeding of penetration may be due to the different host insects and strains of nematodes used (Grewal et al., 2001; Vashisth et al., 2013). After 12 hours, although IJs can penetrate the insect's body through various routes, less penetration occurs through the head, and a higher percentage of IJs, regardless of nematode species and the period of bias of the cuticle, can be penetrated through the cuticles (Hallem et al., 2011; Strong et al., 1996). Eidt and Thurston (1995) reported that IJs could infiltrate insects using several routes depending on availability. Many researchers reported that *Heterorhabditis* species penetrated extensively from the cuticle. Therefore, Vashisth et al. (2018) conclude that the species/isolated *Heterorhabditis* collected from northwestern Himalaya primarily penetrates through the cuticle in *Achoria grisella* Fabricius 1794 and has limited oral permeability. With increasing exposure time, a notable rise in the penetration of IJs within all isolates has been observed through the mouth or the cuticle. After 18 hours of nematode exposure, penetration occurred between 57.4 to 60.4 % through the body and 39.6–42.6 % from the mouth (Kutz et al., 2013; Lu et al., 2017). But still, the ratio of nematodes entering through the cuticle is more than 69% (Půža et al., 2010; Simões & Rosa, 1996).

**An environment conducive to the better performance of nematodes against insects**

Numerous entomopathogenic nematode species have been identified and effectively used commercially in forestry, medical entomology, and agricultural pest management. Suitable microenvironments exist in which EPNs coexist, such as the bottom of rivers, soil pore spaces, and insect hemocoel (Hua et al., 2009; Smits, 1996). Because they have a wide range of hosts, EPNs interact with various hosts in their natural habitats, making them excellent biological control agents. The climate and kind of soil can have a significant impact on how EPNs interact with insects (Glazer et al., 2001; Shapiro-Ilan et al., 2006). Both soil texture and moisture content are significant. Additionally, ecological constraints may impede the insect host's ability to become infected with EPNs (Filgueiras et al., 2017; Hua et al., 2009; Millar & Barbercheck, 2002). Free-living infectious juveniles should be allowed to live on the soil until conditions improve once more in the absence of a host or during unfavorable seasonal conditions like drought. Juveniles infected with free-living status ought to be allowed to live unhindered lives and be able to withstand desiccation brought on by lower soil moisture content (Helmberger et al., 2017; Ignoffo, 1992). Therefore, it cannot be successfully inferred that entomopathogenic nematodes will be effective as biological control agents after they have been parasitic. Nematodes’ ability to move is one of the factors that can lead to decreased infection rates. For instance, a parasitic group of nematodes that couldn't swim could not feast on black flies (Kahel-Raifer & Glazer, 2000; Kaya & Koppenhöfer, 1996). To comprehend how nematodes interact with insects in the natural world, it is crucial to consider these elements. This identification can help contribute to the progression of effective entomopathogenic nematode production and introduction.

**Biology of Entomopathogenic Nematodes**

The life histories of the two genera, *Heterorhabditis* and *Steinernema*, are comparable. Before the eggs hatch, the
mated female of EPN typically lays them up to the second stage of juvenile development (J2). To prevent confusion with the immature stages of insects, it is best to introduce them as juveniles instead of larvae. Every stage molts and feeds to become the subsequent stage; for example, the J2 stage molts to become the J3 stage, which is the adult stage (Fig 1) (Patricia Stock & Goodrich-Bairy, 2012). Reproduction of EPN takes place in various forms, which include amphimictic, hermaphrodite, or parthenogenetic or have alternate gamogenetic and parthenogenetic, and those that reproduce as an amphimictic need male and female; therefore, the adult males are not infective and die post mating, the other only the presence of females is sufficient (Shapiro-Ilan et al. 2017). The existence or absence of important organs or structures, particularly the reproductive and digestive systems, can be used to distinguish between a juvenile's several developmental stages. Therefore, in order to aid readers in identifying each of these stages, even if the morphology of immature stages differs throughout nematode groups, we would like to provide a brief overview of the morphology of immature stages (J1eJ4) of EPN. EPN eggs are oval (average 50-30 mm) and growing a juvenile have mature eggs. J1 usually grows inside the eggs in most of the EPN (Weischer, 2000). This stage is small and transparent in appearance, usually between 10 and 20 mm long, and cannot be seen by a conventional microscope. The body of the J2 is somewhat transparent and has a body length of 250-350 mm and a diameter of 25-30 mm.

At this stage, the digestive system is more or less fully formed. Stoma, mouthparts, and esophagus, in particular, are well-differentiated (Stock & Goodrich-bairy, 2012). Observing the mouthpart at this stage can help distinguish Rhabditids from EPN taxa. At J3, the digestive system is fully developed, and the intestine is very dense because of the presence of preservative material which is approximately 1.5 to 3 times larger than J2. Compared to age J3, the fourth stage of juvenile (J4) is characterized by a relatively long body, depending on the type of EPN species from 100 mm or more (Dillman & Sternberg, 2012; Vashisth et al., 2013). The development of the reproductive organs is remarkable, and the sexes can be identified. Gonads usually look darker than other organs. Female ovaries and uterus can be distinguished from the male testis. In females, the gonads have a sigmoid shape, especially in the middle of the body, while in males the gonad have a direct shape (Patricia stock & Goodrich-blairy, 2012).

**Fig.1. The entomopathogenic nematode’s biology**

**Penetration & symbiotic of bacteria growth**

Nematodes and symbiotic bacteria cooperate to defeat the host insect’s immunological response once they have
One of the most crucial elements in the control of insect pests is the use of entomopathogenic nematodes (Heterorhabditis and Steinernema). When food is emptied into the insect cadaver, an infectious juvenile (IJ) enters the insect's hemocoel because they only have one shot to release them. Insect hemocoel weights include low molecular weight, heat component, and protease resistance. It encourages the discharge of symbiotic bacteria and the proliferation of IJs. Another Rhabditidae member has evolved as a potent biological control agent since 1995 (Jaffuel et al., 2016; Negrisoli et al., 2010; Perry, 1999; Shapiro-Ilan et al., 2012). Slugs and snails have recently been managed with the help of *Pharathorhabditis hermaphroditis*. This species is not linked to any specific bacterium, unlike species *Heterorhabditis* and *Steinernema*. It has been demonstrated that slugs' pathogenicity primarily depends on the type of bacteria they eat and that they may feed on and multiply a wide variety of bacterial species (Grewal et al., 2001). It is possible to use *Phasmarhabditis hermaphrodite* as a model to understand how the ancestors of *Steinernema* and *Heterorhabditis* acquired the harmful bacteria of the insect and evolved defense mechanisms to guarantee exclusive distribution of their symbionts (Batalla-Carrera et al., 2010; Georgis, 2018; Kim et al., 2015).

**Environmental considerations**

Analyzing the environment in which EPNs will be utilized and the elements that could significantly increase the risk of infection is essential. Numerous formulations previously used include polyether polyurethane sponges, clay, bait, activated charcoal, and anhydrobiotic nematodes (Hua et al., 2009; Millar & Barbercheck, 2002). Nematodes must be used to control the final consumer to be regarded as a successful and efficient pest management tool in inappropriate conditions because if they die before use, there will be no point in trading them (Helmerger et al., 2017; Ignoffo 1992). Knowing the ecology of nematodes and the effects of environmental conditions on their activity and infection are crucial for developing effective storage and preparation strategies that will sustain nematode survival in the face of increased infectivity (Grewal et al., 2001; Kaya & Koppenhöfer, 1996). In addition, a good formulation strategy involves determining what kind of infectious children endure.

Consequently, a great deal of study may be done to ascertain which kind of formulation causes an increase or decrease in infection. This is why evacuation analyses are important since they offer a wealth of information. Formulas and formulation materials are also crucial for the long-term viability of EPN (Smits, 1996). The dynamics and composition of the EPN population are severely constrained by their unique life cycle. The capacity of newly emerging insect Japanese (IJs) to disperse and feed themselves until they find a new host is essential to their ability to eliminate insect infestations (Hallem et al., 2011; Shapiro-Ilan et al., 2006). Occurrence, mobility, distribution, and stability of EPNs under the influence of several factors such as intrinsic factors like behavioral, physiological, and genetic characteristics, biological nature, as well as external abiotic variables including temperature, soil moisture, soil texture, soil pH, and UV radiation. These are examples of Intra- and non-specific competitors, predators, parasites, and pathogens. One of the most crucial elements influencing nematode movement is soil moisture since EPN requires a water film for efficient propulsion and dispersion. IJs typically travel through the water film that covers the intermediate spaces in the soil. The nematode's movement is restricted when the water film is extremely thin (in dry soil) or when the intermediate
gaps are filled with water (in saturated soil). Furthermore, the relationship between soil moisture and soil texture in a given soil is established by the interaction of soil particle size and organic matter content (Georgis, 2008; Weischer, 2000). Smaller soil pores typically result in a decrease in nematode motility. Tiny soil pores restrict the amount of oxygen that affects EPN activity and survival, particularly when combined with high soil moisture.

Consequently, low-water, fine-textured soils should have less of an impact from nematodes on insect pests. EPN may use a range of indicators, including temperature, vibration potential, and different inorganic and organic compounds from the hosts, to identify the appropriate hosts. Over the past 20 years, it has become increasingly clear that EPN also relies on signs made from plant roots (Dillman & Sternberg, 2012; Vashisth et al., 2013). These plant-derived odors probably play an important role in combating CO2 gas in the direction of an organism toward or away from the source of stimulation in EPN chemotaxis. Belowground escapes are predicted to operate on scales smaller than aboveground escapes due to poor emission and their interaction with soil particles, which prevents them from spreading in the soil. Previous research has demonstrated that, in circumstances of water shortage, the release of Eβc happens through the gas phase in the sand rather than the aqueous phase. Nevertheless, research has also demonstrated that in artificial sandy soils, the spread of Eβc is slightly constrained (Shapiro-Ilan et al., 2013; Smits, 1996).

Application
The use of entomopathogenic nematodes is possible with almost all agricultural or gardening tools, such as aerial sprays, pressure sprayers, mist blowers, and electrostatic sprayers (Georgis, 2018; Negrisoli et al., 2010; Shapiro-Ilan et al., 2012). The culture system determines the equipment used for EPN, and in each instance, there are several considerations, such as agitation, volume, nozzle type, recycling time, pressure, spray distribution pattern, and system environmental conditions. Ensuring proper stimulation is crucial throughout the application. Using handling tools like backpack sprayers or water cans may be useful for small regions (García-del-Pino & Morton, 2010; Jaffuel et al., 2016; Kim et al., 2015). For application, if EPN in large plots is a proper sprayer apparatus as well as a boom sprayer, it must be considered. Applicants can definitely use alternative techniques, such as the microjet irrigation system, subterranean injection, or bait (Carrera et al., 2010; Georgis, 2018).

The aqueous solution may contain a variety of entomopathogenic nematode formulations, such as clay, polyurethane sponge, activated charcoal, alginate and polyacrylamide gels, peat, vermiculite, and water-dispersible granules. The success of biotic factors depends on several successful applications. Among the most crucial elements for EPN is that the nematodes match the specific target pest (Negrisoli et al., 2010; Shapiro-Ilan et al., 2012). Factors that should be considered in selecting the suitable nematode are as follows: host finding, virulence, in some cases, persistence, and tolerating the environment (Carrera et al., 2010; Perry, 1999). Also, very important for effectiveness; typically, EPNs need to be administered to the soil at a rate of at least 2.5 × 10^9 JJs per hectare (= 25 / cm²) or more. The amount of use may depend on the type of target pest or, in some rare cases, a lower rate. Recycling potential must additionally be taken into account (Bajc et al., 2017; Ebssa et al., 2006; Fenton et al., 2000; Perry, 1999). In general, the nematode population will be high enough, given appropriate environmental conditions, to effectively reduce pests for two to eight weeks following administration. Seasonal reuse is, therefore, frequently required. Nonetheless, across a number of seasons or years, successful control has occasionally been documented (Koppenhöfer et al., 2002; McCoy et al., 2000). On EPN applications, biotic agents may have favorable, unfavorable, or neutral impacts. Nematode infections and predators such as phages, nematophagous fungi, bacteria, protozoa, nematodes, mites, and others are examples of antagonists. It has been demonstrated that morphological relationships exist with other soil species, such as isopods, mites, and earthworms (Imperiali et al., 2017; Vashisth et al., 2013). It has been documented that entomopathogenic nematodes interact together with various entomopathogens, including Paenibacillus popilliae Dutky 1941 Bacillus thuringiensis, Metarhizium anisopliae, and Beauveria bassiana. Depending on the species of nematode and its relative timing or application, the connection between nematodes and various other entomopathogens can change. Several abiotic factors have a critical role in applying EPNs, including sufficient relative humidity and soil moisture, protection from UV rays, and temperature (Dillon et al., 2008; Lacey et al., 2015).

Environmental obstacles like UV and desiccation that lower survival and efficiency have significantly limited the use of EPNs for underground pests; as a result, biocontrol success is more likely to occur when EPNs may be applied to soil or cryptic habitats. Furthermore, because UV light damages nematode treatments, it is recommended to apply it subcutaneously to avoid UV exposure or in the early evening or morning. Moisture is necessary for the survival and migration of EPN in soil applications, but too much moisture might limit mobility and rob it of oxygen (Ali & Wharton, 2013; Parkman et al., 1994). Therefore, irrigation is recommended to
maintain sufficient moisture. The optimum moisture content will vary based on the kind of soil and nematode species. Between the species and strains of nematode may be desirable for infection and reproduction. While some nematodes like *S. feltiae*, *H. megidis*, and *H. bacteriophora* are more resistant of colder temperatures, others—like *S. glaseri*, *H. indica*, and *S. riobrave* are not as heat-tolerant. It is also important to the application for below-ground or surface soil parameters (Dillon et al., 2008; Vashishth et al., 2013). Nematode survival and mobility are impacted by soil texture. Higher clay soils generally restrict nematode migration and may lessen aeration when compared to lighter soils; these factors combined may result in decreased nematode survivability and efficiency (Ebssa et al. 2006; Fenton et al. 2000). Therefore, some exceptions have been reported to this process. Soil pH can affect the natural distribution of EPN. Soils with a pH of 10 or higher are probably not suitable for EPN application whereas EPNs are not significantly harmed by the range of 4–8 (Jaffuel et al., 2016; Negrisoli et al., 2010; Shapiro-Ilan et al., 2012).

**Registration and Regulations**

At present, there is no order or coordination regarding the emergence, discharge, and marketing of nematodes that are entomopathogenic. Due to the unique characteristics of the nematode-bacterium complicated they are regarded as microorganisms in certain countries and most considers microorganisms and anyway are regulated differently. Nematodes are multicellular creatures and should not be categorized as microbes, according to extensive study conducted by the Organization for the co-operation and development of economies (OECD) and the European Commission's Collaboration in the Field of Science and Technical study (COST) (da Silva et al., 2013; Koppenhöfer et al., 2003; Garcia-del-Pino et al., 2018). Therefore, if the newly introduced species are non-native nematodes, they must comply with the following recommendations and adhere to most of the provisions Following the FAO Code of Conduct for biological control acquisition and distribution (FAO 1996): entomopathogenic nematodes must be identified through morphological characters, either by DNA analysis or both, by a reputable laboratory; the collected samples should be at least frozen in the laboratory to improve DNA analysis in the future; In the first step, the target pests are identified and then the nematodes are applied to it; Before the entomopathogenic nematodes are released, it is best to identify species of its and use them on identified insects, as well as other control methods to justify a release; Any nematode identified before use is best used professionally or in accordance with the principles of the Convention on Biological Diversity; Information on the origin, known dissemination, and potential domain of the exotic entomopathogenic nematode, and its safety for the user should be provided; it is very important to have the opinion and approval of a specialist based on the data at hand regarding the potential consequences of non-target organisms is preferred (Ebssa et al., 2006; Georgis, 2018; Koppenhöfer et al., 2003; Shapiro-Ilan et al., 2006; Niekerk & Malan, 2014).

**Commercial assessment**

Entomopathogenic nematodes must meet numerous requirements for acceptable efficacy before breeders will accept them as pest control agents (Ignofo, 1992; Raifer & Glazer, 2000; Kim et al., 2015). Factors such as handling, cost, mixing, durability, compatibility, coating, competition, and profit margins for distributors and manufacturers have had a part in nematodes' inability to enter certain markets or capture a sizable portion of the market. Nowadays, most markets are restricted to particular insects, including citrus fruits, ornamentals, and grasslands (Fenton et al., 2002; Jaffuel et al., 2016). Unfortunately, for the reason of the sensitivity of insects, biology, and/or behaviors, many insects in the product labels of certain businesses have incorrect goals, for example, cucumber beetles, corn rootworms, carrot weevils, flea beetles, wireworms, beach flies, imported fire ants, and root maggots for nematodes (Shapiro-Ilan & Gaugler, 2002; Weischer, 2000). These insects dominate the market for pesticides. Predictive control is essential for nematode-based goods to enter the market (Arefin & Dobes, 2014; Dillman et al., 2012; Lello et al., 1996; Society, 2012). Biological control faces great intellectual difficulty today in reaching predictions due to the complex interplay of biotic and abiotic components. The hosts in which infection and optimal development occur vary among species of nematodes or strains, despite the fact that worms can be successfully infected and produced in a wide variety of host species (Hominick et al., 1996; Kaya & Koppenhöfer, 1996; Lortkipanidze et al. 2016). Consequently, screening a variety of nematode species and strains against the host is crucial to the setup of any management strategy. When creating the control, consideration must also be given to the nematode's behavior and biology, as well as the target's host and the surrounding environment (Lacey and Georgis, 2012).

**Conclusion**

The emphasis is on the need to gather fundamental knowledge about the ecology, genetics, physiology, and behavior of these types of nematodes because it is frequently unknown if they will be successful in controlling insect pests, especially in soil environments. EPNs (especially *Steinernematidae* and *Rhabditida: Heterorhabditidae*) have shown promise as effective biocontrol agents for soil-dwelling insect pests and have therefore attracted widespread commercial attention. The above-mentioned biological control agents have many
advantages such as high virulence, host-seeking ability, ease of use, ease of production, the exception of registration in many countries, and mammalian safety. In addition to having a large variety of hosts, EPN also contains a few widely available chemical insecticides, compatible with a wide range of other control agents, and long-term prep and storage. Thus, the efficiency of applied EPNs can be impacted by both biotic agents, such as EPN predators, pathogens, and other soil organisms, as well as abiotic variables, such as UV, temperature, soil moisture, and relative humidity. According to a recent EPN report, it can lower the nematode population that parasitizes plants.

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