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Entomopathogenic nematodes: possibilities in modern forestry

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Declaration

This study, “Entomopathogenic nematodes: possibilities in modern forestry”, is my own work, and all sources have been cited and acknowledged with full references.

March 13th, 2021, Prague

Peter Morgan

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Acknowledgement

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Abstract

The use of Entomopathogenic nematodes (EPNs) as biological control agents have recently received much attention. This is due to their ability to infect and kill a wide variety insect pest of economic importance and are also environmentally friendly. However, the efficacy of EPNs in the forest ecosystem is affected largely by biotic and abiotic factors. Temperature, moisture, UV light, soil aeration, soil type and carbon dioxide level are some of the abiotic factors that influence the performance of EPNs. Moisture was found to be the most important factor for the survival and efficacy of these parasites. Two main methods of production are known up to date. The in-vitro and in-vivo methods, each having its own advantages and disadvantages. Most ENPs are produced commercially by the in-vitro method because it is cost effective and does not require much expertise. It has been established that the efficacy of ENPs improves when they are combined with other known chemical pesticides. In the case of forest pest management, it would be necessary to combine with certain pesticides considering the high desiccation rate for EPNs.

Keywords: Entomopathogenic, parasite, forest, pesticides, moisture

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1.0 Introduction

Nematodes are among the multicellular organisms that exist in large numbers on earth. There are more than 80, 000 species that have been described in different scientific studies. The number of plants destructive nematodes is small. Sharma et al. (2011) state that plant-parasitic nematodes that have been studied are not less than 2,500. Entomopathogenic nematodes (ENPs) is a class of thread warms that cause death to insect. The name entomopathogenic, *entomon* in Greek, means insect while pathogenic means causing diseases (Kulkarni et al., 2008; Sharma et al., 2011). They are microscopic, soft-bodied, non-segmented, and soil-dwelling worms parasitic to insects. They live naturally in soil and locate their host in response to carbon (IV) oxide, chemical cues, and vibration (Dara, 2017; Kulkarni et al., 2008) and belong to the phylum Nematoda (Gozel & Gozel, 2016; Stoleru & Sellitto, 2016).

In the Czech Republic, soil samples were collected isolate entomopathogenic nematodes from different habitats. Steinernematids were found in 61 of the 87 sampled locations (70.1%). Notable amongst them were *Steinernema. kraussei, Steinernema feltiae, S. affine, S. intermedium, S. bicornutum,* and two other species. *S. kraussei* was found to be more prevalent in sawfly areas (Mráček & Bečvář, 2000). EPNs of the Heterorhabditidae and Steinernematidae families are obligate parasitic insects that are used as biological control agents for economically important insect pests. The two well-known and mostly utilized genera are Heterorhabditis Poinar, 1976, and Steinernema Travassos, 1927. The aim of this study is to provide an overview of existing knowledge of these nematodes and their potential to be used in reducing insect pest population in forestry as it has been successfully used against agricultural insect pests.

2.0 Objective of the thesis

The of goal of this work was to write a review of used nematodes in the biocontrol of main central European forest pests based on existing scientific literature and knowledge.

3.0 Methodology

The methodology used for this study was a literature review. A detailed review of articles on nematodes infestation in forests and management efforts was conducted and many articles were obtained. The research materials were searched and selected based on the content needed to develop the thesis. Articles on mass production of Entomopathogenic Nematodes (EPNs) were searched and analyzed. Other keywords used to help in the search of research materials included Plant Parasitic-nematodes infestation in forests, classification of EPNs, Biology of EPNs, impacts of EPNS in forest trees, combining EPNs with other chemical pesticides, factors influencing distribution and performance of Nematodes in forests, and mass production of EPNs. The variation of keywords was to increase the search results and get resources for each information that had to be covered. There was no restriction on the time frame of the resources. Articles were considered admissible for research if it contained the relevant information as outlined by the search keywords. However, only resources written in the English language were considered and there was no geographical restriction and/or limitation in the selection of resources. Studies and articles by scholars from different parts of the world were considered. Only peer-reviewed articles and books were admissible; website materials were not considered. Both primary and secondary sources were admissible.

4.0 Literature Review

4.1 Classification of Entomopathogenic nematodes

There are many classifications of Entomopathogenic nematodes. Sharma et al. (2011) state that there are close to 20, 000 known species in phylum Nematoda. However, only two families are extensively studied; those belonging to the family of *steinernematidae* and *heterorhabditidae* (Gozel & Gozel, 2016). It is known that because of their symbiotic association with bacteria, nematodes in these families are very lethal (Sharma et al., 2011). The family *steinernematidae* has two genera, *Steinernema* Travassos and *Neosteinernema* Poinar (Gozel & Gozel, 2016; Stoleru & Sellitto, 2016). The latter has only one species of *Neosteinernema longicurvicauda* (Price, 1992) that is distinct from the termite *Recticulitermes flavipes.*

The family *Heterorhabditidae* contains one genus, the *Heterorhabditis* Poinar (Kulkarni et al., 2008; Stoleru & Sellitto, 2016). Until to date, there are 100 valid species of *Steinernema* and 21 valid species of *Heterorhabditis* that have been studied and described in different parts of the world (Bhat et al., 2020). Although they are not closely related, these organisms share some important life histories. Since they have a symbiotic relationship with bacteria, they are categorized as pathogens. Entomopathogenic nematodes are commonly symbiotically associated and connected with bacteria belonging to the family of Enterobacteriaceae. However, as both are different, they are carried by different bacteria. The host bacteria for Steinernematidae belong to genus *Xenorhabdus* and the one carried by *Heterorhabditidae* is a species of genus *Photorhabdus* (Gozel & Gozel, 2016; Stoleru & Sellitto, 2016).

4.2 The Biology of Entomopathogenic Nematodes (Life Cycle)

The life cycles of entomopathogenic nematodes (further EPNs) are completed within several days (D. I. Shapiro-Ilan et al., 2006). As stated before, of all the classifications of nematodes known, only the life cycles of two families are extensively studied because of their economic importance (Dara, 2017). The amount of data available about the two families' life cycles is growing at a rapid rate. According to Stoleru & Sellitto (2016), Heterorhabditis and Steinernematids have similar life cycles, starting from the second generation. During the first generation, there are significant differences in their life cycles. *Steinernema* species are described as being amphimictic meaning that they cannot reproduce successfully in the absence of males and females. On the other hand, *Heterorhabditis* species can reproduce in the absence of conspecifics because they are hermaphrodites. In the second generation, both nematode genera reproduction follows amphimictic reproduction.

According to Devi (2018) the life cycle of EPNs has three main stages: egg stage, four juvenile stages, and adult stage. The infection cycle begins at the third phase of juvenile stages. In other words, the life cycle is initiated by the third stage, which is comprised of Infective Juveniles (Devi, 2018). According to Devi (2018), only the third juvenile stage is an infective juvenile (IJ) that can survive freely in the soil for several weeks without feeding and/or infecting a host. For this reason, the third stage, IJ, is the one that is used in biological control efforts. It is normally encased in a double cuticle that has closed mouth and anus (Devi, 2018). Once a host has been identified and/or located, the nematode gets inside the insect's body via natural body opening including anus, mouth, spiracle, or cuticle (D. I. Shapiro-Ilan et al., 2006).

Once inside the host's body, infective juvenile releases cells of an associated mutualistic bacterium from their intestines into the haemocoel (Stoleru & Sellitto, 2016). Devi (2018) states

that an infective juvenile can carry up to 2000 cells of its symbiont bacterium. The bacterium, in turn, replicates rapidly insides the host's hemolymph. The Nematode offers shelter to the bacterium that destroys the insect host and provides nutrients to it. The bacterium also protects secondary invaders from coming into contact with the cadaver of the host. The infected host does live beyond 48 hours after infection. Within the cadaver, both the nematode and the bacteria feed on the host and replicate for many generations. The larvae grow and mature in adult nematodes (D. I. Shapiro-Ilan et al., 2006).

Nematodes continue reproducing until the time food resources become a problem. The infective juvenile of Steinernematids can develop into either males or females but heterorhabditids develop into hermaphrodites, self-fertilizing organisms. When the host's food supply becomes depleted, the nematode transforms into IJs, which have been modified to withstand the outside world. Although there could be some more generations inside the cadaver, the majority of IJs are released into the environment to look for other hosts and infect and continue with their life (D. I. Shapiro-Ilan et al., 2006). When they leave the internal environment to look for new hosts, they normally carry with them the inoculation of mutualistic bacteria, obtained from the internal environment (D. I. Shapiro-Ilan et al., 2006). As mentioned before, the life cycles of nematodes last for a few days. The reproduction and growth of nematodes depend on conditions established by the bacteria in the host cadaver.

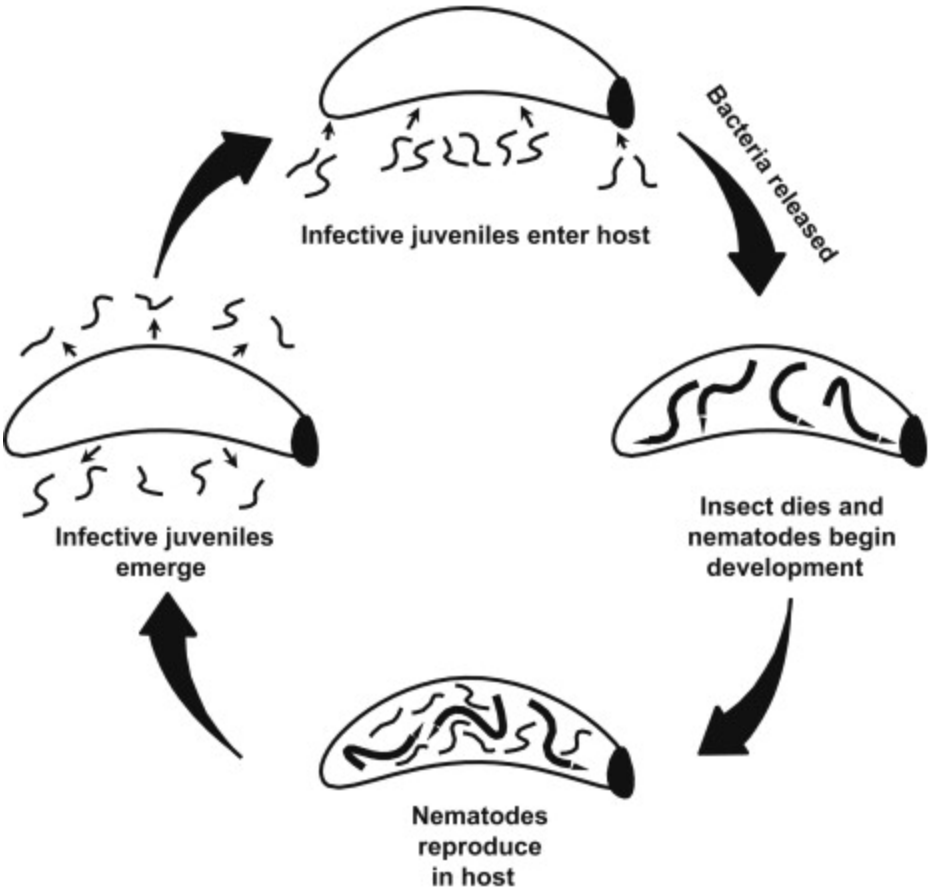

Figure 1. A general lifecycle of EPNs.

Lifted from (D. I. Shapiro-Ilan et al., 2014)

4.3 Mass production of Entomopathogenic Nematodes

For successful and economic usage of EPNs in plant protection, large scale production is necessary. The issue of EPN mass production has been studied widely by several researchers. According to Grewal et al. (2001) and (D. I. Shapiro-Ilan et al., 2014), ENPs can be mass-produced using two major methods; ***in-vivo*** or ***in-vitro*** and the production takes place in the laboratory. The two methods have distinct advantages and disadvantages with respect to economies of scale, production cost, technical know-how needed, and quality of the product. In Vitro method can either

be in solid or liquid culture (D. Shapiro-Ilan & Dolinski, 2015). Generally, Devi (2018) notes that the mass production of EPNs has evolved from the first large scale in vitro trial (solid media culture) in 1940 by Glaser, the in vitro production efforts by Dutky et al.,1964, the three-dimensional solid media in vitro process championed by (Bedding, 1984) and finally to in vitro liquefied fermentation (Friedman, 1990).

4.3.1 In vivo method.

Grewal et al. (2001) note that the *in-vivo* method is the easiest process producing EPNs in live insect hosts because it does not require sophisticated technology, requires the least capital to get started, and entails the use of surrogate hosts. The wax moth, *Galleria mellonella* larvae (Linnaeus,1758) are widely used as surrogate hosts because they are commercially available. The general approach in this method as noted by Shapiro-Ilan et al. (2012) is a system that relies on shelf and tray processing. The in vivo production method is based on a white trap that does well in the juvenile's stage that is characterized by natural migration from the host cadaver. The approach is characterized by inoculation, harvesting, and concentration as well as decontamination. Shapiro-Ilan et al. (2012) say that insects, hosts, are inoculated, on a tray or dish that is lined with an absorbent paper or another material (soil or plaster of Paris) that allows infection by the nematode. This method allows for mass production of EPNs to the tune of between $0.5x10^5$ and $4x10^5$ infective juveniles for every larva, although it does depend on the species of nematode used (Grewal et al., 2001).

After two to five days, the infected insects are moved to the white traps. (Han & Dolinski, 2012) state that if infections are left to progress for too long before they are transferred, cadaver can easily rupture and increase harm to the reproductive nematodes. The yield for in vivo culture depends on several factors including host density and nematode dosage (Han & Dolinski, 2012).

If the dosage is too low, the host mortality is also low. If the dosage is too high, there can be failed infections because of competition from secondary invaders. Therefore, for proper and maximum production, (Han & Dolinski, 2012) recommend the application of intermediate dosages. Overcrowding hosts can result in competition for air, oxygen and consequently builds up of ammonia. Likewise, an optimum host density must be matched with proper inoculation rate for maximum yield. Also, the performance of this method can be affected by environmental factors including temperature, aeration, and humidity. In the United State, in-vivo technology for nematode production has been widely used (Grewal et al., 2001) colleagues note that in the past years, a cottage industry that utilizes the in-vivo technology for mass production of nematodes for sale has emerged and it is popular in-home lawn and garden markets. The major short-coming of in vivo method, according to Grewal et al. (2001) and Shapiro-Ilan & Qiu (2014) is a lack of economy of scale because of the cost of equipment, labor, and insects, which increases linearly with production capacity. Another issue with in vivo production method is the potentiality of biological variation.

4.3.2 In Vitro method.

Shapiro-Ilan & Qiu (2014) state that it requires the largest capital investment to begin but it does payback in the long run as it offers the greatest economic efficiency. In vitro culturing, EPNs are introduced in the pure culture of their symbiont in a highly nutritive medium (non-living medium) (Devi, 2018). The medium uses sterile ingredients to avoid unnecessary and unwanted bacterial contamination. Besides, a non-living media helps retain the fundamental symbiotic bacterium for the nematode while at the same time providing nutrients for growth. Devi (2018) states after sterilization of the medium, inoculation with bacteria follows the addition of nematodes. After two to five weeks, nematodes are harvested in water. According to Devi (2018)

and Shapiro-Ilan et al. (2012, 2014), in Vitro mass production of nematodes, particularly the *Steinernema glaseri* was first tried in the United States in control and management of *Popillia japonica.* It was done by Bedding in 1984 and it came to be described as solid culture. In solid culture, "nematodes are cultured on a crumbed polyether polyurethane sponge impregnated with emulsified beef-fat and pig's kidneys along with symbiotic bacteria" (Grewal et al., 2001). The first time in vitro solid media was used, between $6x10^5$ and $10x10^5$ infective juveniles for every gram of the medium, were obtained. The in vitro method using solid culture has been used widely in the USA, China, and Australia.

In research completed by Friedman in 1990, it was reported that the solid culture method was and is economically feasible as it can yield up to $10x10^{12}$ nematodes every month (Friedman, 1990). The liquid fermentation technique for mass production of nematodes was also reported in Friedman's 1990 experiment study. According to Grewal et al. (2001), the liquid fermentation technique has the capability of producing up to $50x10^{12}$ infective juveniles every month. In addition, Grewal and colleagues state that the recent improvements in liquid culture technology have resulted in improvement in quality as well as total yields. For example, the current yield of *S. carpocapsae* in in-vitro liquid culture is not less than 2.5x 10^5 infective juveniles for every gram of culture (Grewal et al., 2001).

Shapiro-Ilan et al. (2012) note that the mass production of EPNs in vitro solid culture can be affected by several factors including nematode inoculation rate. That is, infective juveniles available per unit of media can influence yield in some strains of nematodes. Also, it is important to note that culture time is indirectly related to temperature and therefore, it should be optimized to get maximum yield depending on the species or strain. Increasing the size of inoculum can stimulate the growth of nematodes and decrease the culture time. However, although longer culture

can boost yields, nematode mortality may increase with time as well. The culture time must be determined by considering several factors including the cost of diminishing returns and space available. The composition of the media is another potential factor that can affect production capacity and level. Shapiro-Ilan et al. (2012) state increasing the quality and quantity of lipids can increase the yield of the nematode. Also, the quality and quantity of salt and proteins can affect nematode yield in solid culture. The in vitro technology is characterized by significant improvement as noted by Shapiro-Ilan & Qiu (2014) because it utilizes large fermenters that allows the production of large quantities of EPNs for commercial purpose.

4.4 Survey of nematodes species in forest pests using ENPs in forest pest management

Entomopathogenic nematodes are extremely lethal to many important insect pests, are nontoxic to nontarget species, and destroy insects in as little as 24-28 hours while operating with their symbiotic bacteria, as opposed to days or weeks with other biological control agents. Their infective juveniles (IJs) have been shown to withstand a variety of chemical and biological agent exposures for short periods of time. Since these nematodes are compatible with a wide range of agrochemicals, they are a cost-effective pest control alternative.

ENPs from the *Steinernematidae* and *Heterorhabditidae* families have attracted a lot of attention as effective biocontrol agents for insect pests living in the soil. These biological control agents have a variety of benefits, including the ability to seek out hosts, high virulence, ease of processing, ease of application, mammalian protection. They also have a wide range of hosts, are compatible with a number of other control agents, are widely distributed, and can be produced and stored for an extended period of time (Vashisth et al., 2013).

4.4.1 Control of *Hylobius abietis* using ENPS

Hylobius abietis grows under the bark of felled conifer stumps and is found in most of the world's coniferous forest regions, posing a major problem for seedling survival on plantation forestry. EPNs and other bio-pesticides have been tested in aqueous suspension around stumps to attack growing larvae and pupae (Dillon et al., 2006). The free-living host-seeking stage, infective juveniles (IJs) or dauers, invade the host through body openings (spiracles, mouth, and anus) or the cuticle, releasing symbiotic bacteria (*Photorhabdus* sp. in *Heterorhabditidae* and *Xenorhabdus* sp. in *Steinernematidae*) (Harry K Kaya & Gaugler, 1993). The bacteria break down the host tissue, providing a food medium for the growing nematodes to feed on. Only *Steinernema carpocapsae* Weiser has been used to kill pine weevil on a wide scale in Europe. Despite the fact that *Heterorhabditis downesi* Stock, Griffin, and Burnell is not commercially available, previous research has shown that it is more effective against pests (Dillon et al., 2006; Williams et al., 2013).

A single application of nematodes at a rate of 3.5106IJs in 500 ml of water around the base of every stump, equal to 7.5109IJs/ha, is used to control the big pine weevil. Larvae can be infected, killed, and reproduced by three nematode species commercially available in the UK. At 7.5 109 IJs/ha, *Steinernema carpocapsae* and *S. feltiae* developed comparable levels of infection in about 56% of field populations but *Heterorhabditis megidis* had a much lower efficacy (Brixey, 2000). *Steinernema carpocapsae* has been selected as the primary control agent for further trials because it is the simplest and cheapest to produce.

4.4.2 Control of *Ips* spp using ENPs in forestry

Ips spp. nematodes range in size from 0.2 mm to 2.0 mm (*Cryptaphelenchus*) (*Contortylenchus*). Endoparasitic nematodes are commonly found in the body cavities of adults, larvae, and pupae in the case of bark beetle parasites. They suck fluids from the beetle's body cavity, invade the intestine, then molt into adulthood, copulate, and lay eggs in the gallery. They eat the entire fat body and other tissues of the host in some situations. Bark beetles are often infested with endoparasitic nematodes, with infestation rates reaching 50% in some cases (Burjanadze & Goginashvili, 2009). Nematodes, according to (Massey, 1974), are a significant factor in reducing bark beetle populations. Despite the fact that these nematodes hardly ever destroy their hosts, they can alter their behavior by lowering host fertility, survival, and flight activity, as well as delaying swarming (Hoffard & Coster, 1976; H K Kaya, 1984).

4.4.3 Control of *Cephalcia* spp with ENPs

Just two insect species, both belonging to the genus *Cephalcia*, have been found to be infected with *Steinernema kraussei*. *Cephalcia abietis* seems to have a close relationship with the nematode and it was thought to live in close proximity it (Fischer & Führer, 1990). *S. kraussei* and *Cephalcia abietis*, the false spruce webworm, are perhaps the most well-known healthy host-nematode association. *S. kraussei* (and possibly also *Steinernema feltiae*) are the most common causes of mortality of this insect in Austria (Fischer & Führer, 1990), the Czech Republic (Mráček, 1986), and southern Germany (Eichhorn, 1988).

Mracek (1986) discovered 3-20% of a *Cephalcia abietis* population infected with *S. kraussei* in a systematic study conducted between 1975 and 1980. Over the course of a year, the nematode kills 24 to 27% of the *C. abietis* population, according to this report. *C. abietis* infection rates are similar in Germany and Austria. S. kraussei was found to infect *C. falleni* as well, but at much lower concentrations (0.8-0.9 %) (Eichhorn, 1988). *Steinernema carpocapsae* also afflicted populations of the closely related d larch saw fly *C. lariciphila* in the UK. Sawfly larvae were infected at a rate of 3.4-29.4% when foliar sprays of 5,000-

20,000 nematodes/100 cm^2 branch were applied. Sawfly prepupae were infected with 61% and pupae with 17.3% of 200 nematodes/cm^2 applied to the soil.

One year after nematode application, prepupal infection ranged from 4.8 to 14.7%. This nematode's soil applications show that it can regulate sawfly prepupae, which are responsible for 8-15% mortality (R Georgis & Hague, 1981). Certain farming practices can greatly improve the control capacity of naturally occurring populations of entomopathogenic nematodes. After applying magnesium fertilizers to Austrian spruce forest soils, Fuhrer and Fischer found a rise in *C. abietis* nematode infestation. They suggested that raising the soil pH would improve the potential of *S. kraussei* to infect (Führer & Fischer, 1991).

4.4.4 Biological Control of *Melolontha spp*

The larvae inflict substantial and lethal damage to the roots of young plants, while the adults eat the flowers and young leaves of fruit trees and other forest and decorative trees. Many fruit trees and perennial crops are severely damaged in central Europe, especially in Hungary, where integrated fruit production is practiced (Lakatos & Toth, 2006)

Beauveria brongniartii, an entomopathogenic fungus, is the only product currently available that is effective against cockchafer larvae. It is licensed for use in a few countries, including Austria, Italy, and Switzerland, and is sold under a variety of brand names. Unfortunately, Hungary has yet to authorize it for use. However, the Beauveria products is also restricted by higher soil temperatures and in Hungary often reach 27°C which limits the growth of *Beauveria*. Higher temperatures destroy spores, preventing the hyphal network from growing, which is necessary for effective control (Kessler et al., 2003).

Amongst the many ENPs, *Steinernema glaseri* was found to be the most effective nematode species in laboratory experiments for controlling the European cockchafer (Peters & Keller, 1998). *S. glaseri*, on the other hand, is not commonly used in Europe since it is from the United States.

ENPs were used in Hungary to develop a biocontrol product that was successful against European cockchafer larvae. Five strains from the Heterorhabditidae family were chosen, but only one,

Heterorhabditis downesi Strain 267, proved to be highly effective in the soil test. Figure 2 depicts the dose response of this strain. At a dose of 1,000 IJs per gram of soil, Strain 267 caused approximately 90% mortality, and at a dose of 100 IJs per gram of soil, it caused approximately 50% mortality. The ideal temperature was 20°C, but mortality was high at 15°C as well (Lakatos & Toth, 2006).

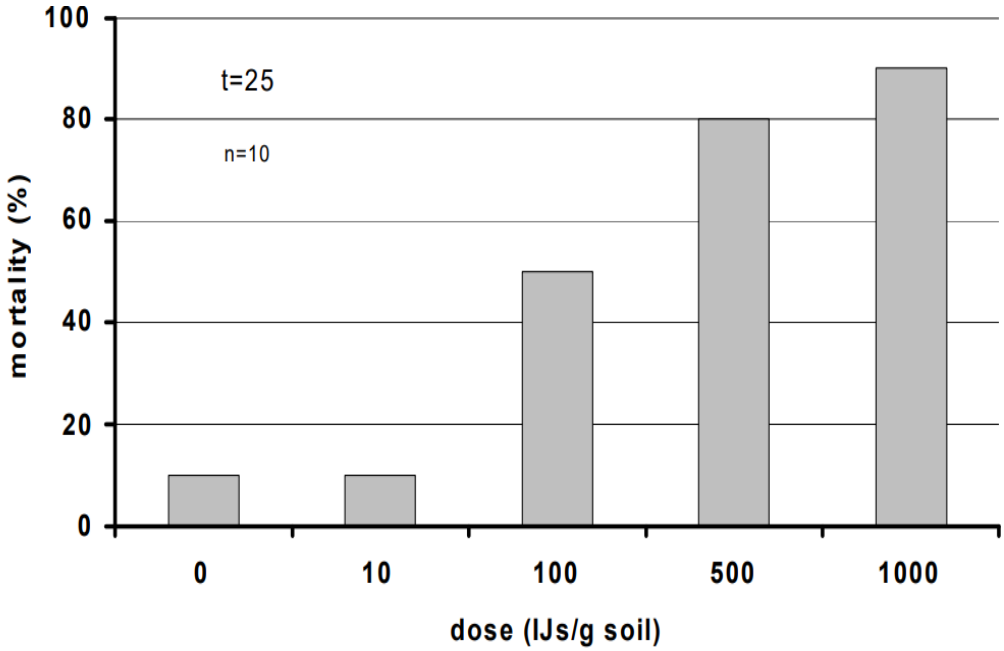

Figure 2. Heterorhabditis downesi Strain 267's efficacy against Melolontha melolontha larvae at various doses

Source: (Lakatos & Toth, 2006)

Table 1. Endoparasitic nematodes associated with Ips species in central Europe

Nematode species	Nematode family	*Ips* species	Location	Country	Reference
Contortylenchus acuminati Ruhm, 1956	Allantonematidae	*I. acuminatus*	hemocel	Germany	Ruhm 1956
Contortylenchus amitini Ruhm, 1956	Allantonematidae	*I. amitinus*	intestinum	Czech Republic, Germany, Slovakia	Ruhm 1956, Weiser *et al.* 2006, Vilagiova 1993
Contortylenchus diplogaster v. Linstow, 1890 *	Allantonematidae	*I. cembrae* *I. sexdentatus* *I. typographus*	hemocel	Czech Republic, Germany, Poland, Slovakia	Ruhm 1956, Tenkacova, Mituch 1986, 1991, Balazy 1966, 1968
Cryptaphelenchus macrogaster acuminati Ruhm, 1956	Aphelenchoididae	*I. acuminatus*	Malphigian tubules intestinum	Germany	Ruhm, 1956
Cryptaphelenchus macrogaster macrogaster (Fuchs, 1937) *	Aphelenchoididae	*I, cembrae* *I. typographus*	Malphigian tubules intestinum	Germany, Slovakia	Ruhm 1956, Tenkacova, Mituch 1986, 1991
Ektaphelenchus typographi (Fuchs, 1930) *	Ektaphelenchidae	*I. typographus*	larvae	Germany	Ruhm 1956
Parasitaphelenchus acuminati Ruhm, 1956	Parasitaphelenchidae	*I. acuminatus*	hemocel intestinum	Germany	Ruhm 1956
Parasitaphelenchus sexdentati Fuchs, 1937 *	Parasitaphelenchidae	*I. sexdentatus*	hemocel intestinum	Czech Republic, Germany	Weiser, Mracek 1988, Filipjev 1959
Parasitorhabditis acuminati (Fuchs, 1937) *	Rhabditidae	*I. acuminatus*	hemocel intestinum	Germany	Ruhm 1956
Parasitorhabditis amitini (Fuchs, 1915) *	Rhabditidae	*I. amitinus*	intestinum	Germany, Slovakia	Ruhm 1956, Tenkacova, Mituch 1987, 1991
Parasitorhabditis obtusa (Fuchs, 1915) *	Rhabditidae	*I. cembrae* *I. typographus*	intestinum	Austria, Switzerland, Czech Republic, Germany, Poland, Slovakia, Slovenia	Ruhm 1956, Weiser, Mracek 1988, Tenkacova, Mituch 1986, 1987, 1991, Balazy 1966, 1968, Andrassy 1983
Parasitylenchus dispar (Fuchs, 1915)*	Parasitylenchidae	*I. typographus*	hemocel	Czech Republic, Germany, Poland, Slovakia, Slovenia	Ruhm 1956, Balazy 1966, 1968, Weiser 1954, 1977, Weiser, Mracek 1988, 2006, Tenkacova, Mituch 1986, 1991

Source: (Grucmanová & Holuša, 2013).

4.5 Formulation and application of ENPs

There are not many rules on how entomopathogenic nematodes should be formulated and implemented to maximize their production, as recently noted (Gan-Mor & Matthews, 2003). While

most biopesticides are currently only applied with traditional agricultural spray equipment (H K Kaya, 1990), developing alternative application methods may be beneficial. The use of a hydraulic spray gun to concentrate nematode suspensions close to trunks, for example, resulted in higher infection rates of codling moth larvae than the more widely used but less concentrated air blast sprayer (Unruh & Lacey, 2001). Battery-operated spinning disc sprayers are a low-cost option for poor farmers in developing countries.

In comparative experiments, spinning discs killed nearly half of the *Plutella xylostella* larvae on cabbage while applying just 9% of the nematodes applied with hydraulic nozzles (Lello et al., 1996), implying that further research on low-volume systems could be economically justified. A novel slow-release system against various orchard pests is to use nematode-impregnated collars mounted around hibernation sites on tree surfaces (H K Kaya, 1984; Nachtigall & Dickler, 1992). When used to fight above-ground pests, the use of pre-desiccated nematode formulations demands extra caution. (Baur et al., 1997) found that unless nematodes were rehydrated for 48 hours prior to use, the effectiveness of a wettable granule (WG) formulation of *S. carpocapsae* against *P. xylostella* was decreased. The implementation method chosen may have an impact on how nematodes should be formulated to achieve the best performance.

(Battisti, 1994) reported that, one month after application, the amount of non-parasitized pre-pupae in the soil in areas treated with SK was decreased by 25.1 percent when compared to untreated areas. There was no mortality from the other nematode strains. The IS 389 therapy reported the greatest decline in the number of pre-pupae four months later (63.4%). The mortality of the pre-pupae caused by SK was lower in application B than in application A. When 100 juveniles of S. feltiae and S. kraussei were applied to the soil before the mature larvae dropped and entered the soil, sawfly emergences were reduced by 56 percent and 36.4 percent, respectively.

When the nematodes are added after the larvae have already prepared their chambers, the effectiveness of *S. feltiae* increases to 32.3 percent. S. feltiae parasitized more females and individuals who were long-term diapausing than *S. kraussei*. The entomopathogenic nematodes *S. feltiae* and *S. kraussei* will reduce the number of pre-pupae of C. arvensis in the soil of Norway spruce forests when introduced before the mature larvae reach the soil, but Heterorhabditis sp. and *S. carpocapsae* cannot (Battisti, 1994).

The use application of nematodes in forestry has resulted in a variety of outcomes, all of which are dependent on the insect's life stage being targeted correctly. Foliar insecticides, such as those used to monitor the spruce bud moth *Zeitraphera canadensis* (Mutuura and Freeman), have had mixed results. Targeting life stages that occur in environments with more nematode-friendly conditions has yielded more promising results. ENPs were injected in a gel suspension into the er nests of the pine caterpillar, *Thaumetopoea pityocampa* (Denis and Schiffermuller), a major pest of pines in the Mediterranean area and the results were encouraging (Triggiani & Tarasco, 2002). Promising results were also reported against prepupae of the web-spinning larch sawfly, *Cephalcia lariciphila* (Wachtl), in Wales (Ramon Georgis & Hague, 1988).

4.6 Commercially available species of ENPs

Twenty-three (23) nematode families have been shown to have parasitic interactions with insects. Seven of these families include organisms that have the ability to regulate insects biologically (Koppenhöfer & Kaya, 2002). Just a few species kill insects, but they are difficult

(e.g., tetradomatids) or costly (e.g., mermithids) to be produced commercially, have limited host specificity against minor economic pests, have low virulence (e.g., sphaeruliids), or are otherwise unsuitable for pest control. Because of their ability to destroy hosts easily, entomopathogenic or insecticidal nematodes in the genera Steinernema and Heterorhabditis are the only insect-parasitic nematodes with an optimum combination of biological control attributes (1-4 days depending on nematode and host species). Just about a dozen species of Steinernematids and Heterorhabditis nematode have been commercialized (Vashisth et al., 2013). The parasitic nematode species shown in Table 2 are commercially available.

Table 2. Showing some commercially available ENPs species

Nematode species	Product formulation	Country
Steinernema carpocapsae	ORTHO Biosafe USA	USA
	Bio Vector USA	USA
	Exhibit USA	USA
	Sanoplant	Switzerland
	Boden Nutz;linge	Germany
	Helix	Canada
	X-GNAT	USA
	Vector TL	USA
S. feltiae	Magent	USA
	Nemasys	UK
	Stealth	UK
	Entonem	USA
S. riobrave	Vector MG	USA
	Bio Vector	USA
S. scapterisci		USA
Heterorhabditis bacteriophora	Otinem	USA
H. megidis	Nemasys	UK
S. carpocapsae	Green commandos	India
	Soil commandos	

Source: (Vashisth et al., 2013).

4.7 Abiotic factors influencing the use ENPs as biological control agent

4.7.1 Temperature

Both (Matlack, 2001) and (Zadji, 2014) state that temperature is a major factor that not only influences the performance of organisms but also their distribution and location in their natural ecosystems. The quality of the temperature needed varies from one species to another. Certain nematode species including *Heterorhabditis indica, S. riobrave, and S. glaseri* operate at high temperatures. (Zadji, 2014) states that they can perform at temperatures ranging from 29°C and more. They are relatively tolerant of heat and therefore, they tend to be found in a hot climate. Other organisms such as *S. felitae, Heterorhabditis marelatus (Liu&Berry, 1996), and Heterorhabditis megidis (Jackson&Klein, 1987)* are only tolerant in cold regions, preferably those that range from 15°C and less.

(Grewal et al., 2001) when studying about the effects of temperature on some species of Entomopathogenic nematodes, found that *S. felitae* infected and established in *G. mellonella* larvae in a temperature range of between 8 and 30°C went to reproduce between 10 and 25°C. *S, riobrave* infected and established in a temperature range of between 10 and 39°C, reproduced between 20 and 35°C. *S. carpocapsae* operates well at a temperature ranging from 20 to 30°C. However, at temperatures between 35 and 37°C, its locomotion and infectivity are impaired. Therefore, the distribution of nematodes in a different forest environment is a factor of temperature (Zadji, 2014). Nematodes will live in areas where the temperature is conducive for them to survive. (Platt et al., 2020) state that nematodes must be kept in aqueous solutions between 4 and 30°C. Most are not tolerant to temperatures above 35°C.

4.7.2 Moisture

Moisture is another essential factor that affects and influences the distribution nematodes in forest soils (Zadji, 2014). Optimum soil moisture is needed for better performance and efficacy of nematodes. The soil moisture influences survival, movement, and pathogenicity of EPNs. However, too much or little moisture is a threat to their survival. When moisture is too much, there can be oxygen deprivation thereby restrict the movement of EPNs. According to (Matlack, 2001), ENPs nematodes cannot survive for long in dry soil. He notes that desiccation reduces the survival ability of nematodes. Therefore, nematodes will not stay in environments where soil moisture is not sufficient to sustain their activity.

4.7.3 Soil texture

Both (Zadji, 2014) and (Matlack, 2001) state that soil texture is critical for the movement and spread of EPNs. Therefore, they will not inhabit areas where soil texture inhibits their movement. On this note, they are likely not to be found in forest areas characterized by heavy clay soils. (Zadji, 2014) states that the movement of nematodes in soil improves with a decrease in silt and clay content. Also, the thickness of organic matter present in the soil is a threat to the nematode movement. Nematodes respire aerobically and therefore low oxygen content in the soil can impact their activity and ultimately survival. (Zadji, 2014) states that nematodes perform better in soil pH below 10.

4.7.4 Ultraviolet (UV) radiation

(Matlack, 2001) states that UV radiation is a threat to EPNs activity and survival. The infective juveniles are susceptible to UV rays and die if they are exposed to the light of around 300

nm. They can only tolerate direct sunlight for only less than an hour. *S. carposcapsae* is susceptible to short UV below 254 nm. However, it can thrive well in longer UV, at least 366 nm. Therefore, it can be effective in biological control involving exposed surfaces. When applying aboveground EPNs, it is important to take note of UV radiation. (Platt et al., 2020) found that application or exposure of infective juveniles to short UV radiations for more than seven minutes reduces their pathogenicity by 95% by the end of the hour. Also, they found that *S. carpocapsae* infective juveniles became inactive after 10 minutes of exposure to UV radiation. *H. bacteriophora* is affected after four minutes. These findings show that the susceptibility of EPNs varies across species.

4.7.5 Biotic Factors

The distribution of nematodes is not only affected by abiotic factors but also by biotic factors. Nematodes, like other organisms, have predators and, pathogens, and competitors and/or antagonists. Microorganism such as nematophagous fungi which are common in many parts of the global soils affect EPNs dynamic. The act as predators and therefore, in areas where they are in large numbers, it is hard to find EPNs in greater populations. Other organisms in the soil can provide positive benefits to nematodes as far as dispersal and movement are concerned. For example, the dispersal of *Steinernema* spp. is said to be increased by earthworms (Zadji, 2014). Further research indicates that some insect hosts allow for the phoresis thereby enhancing dispersal to a greater area than what EPNs can achieve on their alone (Hua et al., 2009). Competition for resources is another factor. Competition between nematodes and other organism influence availability of nematodes in certain locations. Their population is low in areas of great competition and the opposite is true in areas with stable less to no competition.

4.7.6 Impact of CO_2 on Nematode Genera in Forest Soil

The performance of nematodes in the forest is a highly affected level of carbon dioxide in the soil. The diversity of soil nematodes as well as ecological succession decreases in response to higher levels of carbon dioxide. The response to carbon dioxide varies between genera. In other words, the species in different genera respond differently to carbon concentration in soil. (D A Neher et al., 2004) did a study to determine the impact of carbon dioxide on different types of nematodes. The study was conducted in loblolly pine and sweet gum forests in Indices. A similar study was conducted by (Deborah A Neher & Weicht, 2013). They extracted nematodes from roots and soils of sweetgum and loblolly pine forests fumigated with carbon dioxide. The researchers were observing the change in three attributes: biomass, respiration, and abundance of nematodes. They found that elevated carbon dioxide has a variety of effects on various communities of soil nematodes. In the case of an elevated carbon dioxide concentration, the population of nematodes was found to have decreased in both pine and sweet gum forests. In the Loblolly Pine forest, respiration and biomass of nematodes increased with elevation of carbon dioxide but decreased in sweet gum forests (Deborah A Neher & Weicht, 2013).

In both loblolly pine and sweet gum forests, researchers noted that the bacterivores and fungivores had their abundance, biomass, and respiration affected by elevated carbon dioxide. In bacterivores, a decline in abundance, biomass, and respiration was recorded in both pine and sweet gum forests but in latter, the relative change was greater than in pine plantations. The fungivorous nematodes showed a decline in biomass and respiration upon increasing the amount of carbon dioxide in both forests. However, their abundance increased with carbon dioxide elevation. In sweet gum forests, the abundance, biomass, and respiration of predator nematodes decreased with elevated carbon dioxide (D A Neher et al., 2004; Deborah A Neher & Weicht, 2013). In pine

forests, biomass was of predatory nematodes was increased upon fumigation with carbon dioxide while the other two attributes- respiration and abundance decreased. Nematodes in the class of herbivores are not affected by elevated carbon dioxide. There is no change in terms of abundance and, biomass, and respiration when fumigated with carbon dioxide (D A Neher et al., 2004; Deborah A Neher & Weicht, 2013). At Loblolly pine forest, Neher et al. (2004) found that carbon dioxide elevation increases respiration and biomass of the nematode community. Also, the abundance of fungivores and the biomass of predators and omnivores increases. Table three below, taken from the work of Neher et al. (2004) shows how nematodes respond to an elevated level of carbon dioxide.

Table 3.Effects of elevated Carbon dioxide on Nematode communities

	Pine			Sweet Gum		
Index	Ambient ($n = 29$)	Elevated ($n = 32$)	$\%r^2$	Ambient ($n = 36$)	Elevated ($n = 25$)	$\% r^2$
Total number	18·3 ± 3·76[a]	16·6 ± 3·40	52	7·3 ± 0·98[a]	5·7 ± 0·96	68
Total respiration[b]	15·89 ± 3·31[a]	16·83 ± 4·33	44	7·46 ± 1·02[a]	4·53 ± 0·62	65
Total biomass[c]	2900 ± 610[a]	3100 ± 800	31	1400 ± 190[a]	800 ± 120	65
Abundance (%)						
Herbivores	11·8 ± 1·3	12·3 ± 1·3	13	14·2 ± 1·7[a]	10·4 ± 1·3	49
Fungivores	50·3 ± 2·5[a]	50·7 ± 2·1	33	40·7 ± 2·1[a]	46·6 ± 3·2	79
Bacterivores	33·4 ± 2·3[a]	32·1 ± 2·1	38	38·4 ± 2·8[a]	36·4 ± 3·7	70
Omnivores	0·8 ± 0·2	0·9 ± 0·2	20	2·3 ± 0·4	2·7 ± 0·4	29
Predators	3·1 ± 0·4	3·1 ± 0·4	14	2·7 ± 0·3[a]	2·2 ± 0·4	49
Respiration[b]						
Herbivores	2·97 ± 0·91	3·17 ± 0·81	23	2·57 ± 0·57[a]	1·00 ± 0·25	64
Fungivores	3·46 ± 0·91[a]	2·95 ± 0·64	41	1·32 ± 0·19[a]	1·25 ± 0·22	64
Bacterivores	3·37 ± 0·47[a]	3·31 ± 0·70	35	2·01 ± 0·28[a]	1·36 ± 0·21	67
Omnivores	0·25 ± 0·08	0·42 ± 0·13	28	0·38 ± 0·09[a]	0·78 ± 0·09	51
Predators	5·92 ± 1·61	6·89 ± 2·90	21	1·38 ± 0·25[a]	0·49 ± 0·13	46
Biomass[c]						
Herbivores	55 ± 17	59 ± 15	23	48 ± 11[a]	19 ± 4	64
Fungivores	63 ± 16[a]	54 ± 12	41	24 ± 4[a]	22 ± 4	64
Bacterivores	62 ± 9[a]	61 ± 13	35	37 ± 5[a]	25 ± 4	67
Omnivores	5 ± 0[a]	8 ± 3	28	7 ± 2[a]	7 ± 2	51
Predators	109 ± 20[a]	126 ± 53	21	26 ± 5[a]	9 ± 3	46

Source: (D A Neher et al., 2004)

4.8 Biological control of forest pests

4.8.1 EPN targeting different stages of growth in forests pests

EPNs have been used as biological control agents for various plant pests (insects) because of their successful activity against different economically important insect pests (Belien, 2018). While all EPN genera are said to be effective in biological pest control, the nematodes in the genera *Steinernema* spp. and *Heterorhabditis* spp. are the most widely used to control insect pests belonging to the order Diptera, Orthoptera, and Lepidoptera. As mentioned before, they kill insects through the help of a mutualistic bacterium. Examples include *Steinemema carpcocapse, Steineenema felitae, Steinemema kraussei, Heterorhabditis megidis,* and *Heterorhabditis bacteriophora* (Belien, 2018; Lacey & Georgis, 2012). They are widely used because it is easy to produce them in liquid culture. Among other factors, the efficacy of nematode activity is influenced by nematode species, production, strain, storage condition, and persistence in the habitat (Lacey & Georgis, 2012). Also, the susceptibility of the target host is an important factor. The environmental condition is also a factor that influences the efficacy of EPNs. These factors include among others temperature, humidity, soil type, aeration, organic matter content, UV light, and soil salinity (Belien, 2018; Lacey & Georgis, 2012; Zadji, 2014).

Belien (2018) states that the efficacy of EPNs in control of insect pests has been realized in the management of insects such as beetles, butterflies, and flies especially in cryptic and foliar habitats. EPNs are deployed in environments and habitats where chemical pesticide fails. In the forest ecosystem, they stand out compared to chemicals. Besides, the desire for adopting eco-friendly environmental practices has led to the mass production of artificial nematodes. The commonly used EPNs for targeting pests in the forest include EPNs targeting subterranean pest stages and EPNs targeting aboveground pest stages.

4.8.2 EPNs Targeting Subterranean Pest Stages

According to Belien (2018) soil is the most climatologically stable habitat in the natural habitat of nematodes. A lot of subterranean EPNs tend to be isolated from the soil habitats. Many of them have received attention for control with EPNs. Most of the current successful EPN application target subterranean pes stages (Belien, 2018). In most forest ecosystems, soils are natural and excellent habitats for the nematodes. Therefore, with conducive environmental conditions (favorable temperature, proper soil moisture, proper aeration, and susceptible hosts), the control of subterranean pest stages is quite effective (Lacey & Georgis, 2012). However, for better performance, it requires the use of EPNs with a mobile forging strategy. That is, intermediate and cruiser foraging strategies. The advantage of cruisers is that they have a more active movement in soil and deploy distant volatile cues to aid in finding/locating host(s). The ambush foragers remain near the soil surface (Lacey & Georgis, 2012). They find a host by lifting their body into the air to catch any passing host. The root-feeding beetle larvae are the most effective underground ENP targets.

4.8.3 EPN Targeting Aboveground Pest Stages

In the past years, there have been many research developments focusing on the application of EPNs against aboveground pest stages. Platt et al. (2020) note that EPNs have been deployed successfully against insects in the forest in North America, Europe, Japan, China, and Australia. The major aboveground sections include epigeal (soil surface), cryptic (bark cracks, leaf litter, under bark, prop piles, pruning wounds, and nutshells among others), and foliar (canopy/leaves) habitats. In all these types, Belien (2018) notes that both cruisers and intermediate foraging strategies can be successful. However, the most effective EPNs are those with a sit and wait for foraging strategy (Belien, 2018). They are effective for targeting soil and cryptic habitats. Because

of sensitivity to desiccation and ultraviolent rays, EPNs suitable when applied to soils and/or cryptic. In other words, targeting aboveground pest stages can be effective than subterranean targeting. For some insect pests such as *D. abbreviates* larvae that are susceptible to EPNs, the best EPN treatment should be soil-based (targeting those on the soil). Soil targeting treatments are effective because they interact with nematodes when they are entering soil thus resulting in high control. Codling moth is a good example of an aboveground pest targeting EPN. Codling moth has a life stage in cryptic habitat. Although their caterpillars feed pests when they mature they look for cryptic habitats. In 2004, a meta-study was conducted using 136 trials on aboveground applications of *S. carpocapsae* (Arthurs et al.,2004). The researchers, as documented by Platt et al. (2020), found that soil-based stages of insects are the most successful. They noted that the efficacy of EPNs varies depending on the target habitat. It also varies according to location and environmental factors. Studies show that aboveground applications of entomopathogenic nematodes for insect control is normally used to control Lepidoptera, Hymenoptera, Diptera, and Coleoptera. Platt et al. (2020) wrote that the aboveground stages of insects can be easily and efficiently targeted in different macro environments.

4.8.4 Nematode management in forest trees

Forests are important natural resources for the survival of all living things on the earth. Over time, the global forest cover has been diminishing due to natural causes such as pests and fires, as well as anthropogenic-induced causes such as deforestation for timber and the clearing of land for agricultural and settlement purposes. If the world's population grows, the forest begins to disappear. Natural forest makes up 95% of the current cover, with cultivated forest accounting for the remaining 5%. It's worth noting that tropical areas account for 47% of total global forest cover, while subtropical areas account for 9%, boreal areas for 33%, and temperate areas for 11%. For

micro and macroorganisms, the forest flora offers a good living climate. Researchers have researched fungi and bacteria more than entomopathogenic nematodes among the identified forest pathogenic microorganisms. ENPs are present all over the world, and their distribution is affected by a number of factors, both biotic and abiotic. Environmental factors, as well as the vulnerability of the host, play a large role in the development of plant diseases, especially those caused by nematode infestation. Nematodes, as previously mentioned, are soil dwellers that primarily attack plant underground sections (FA0, 2010).

In global forests, nematodes are a growing threat. They've evolved into serious pests, necessitating the most successful control and management strategies to protect forests from their negative consequences. Chemical spraying, biological techniques, and tree cutting rotation have all been used in the forest to combat nematodes. Khan (2012) acknowledges that there has been a lot of work done by researchers to aid the control of nematodes in forests. Forest nematode control has used botanicals, microbial preparations, additives, and cultural processes. Integration and combination of different management methodologies that have proven to be successful in the control of nematodes, especially in a variety of climatic conditions, has led to unprecedented success. Nematologists classify the management of nematodes in the forest as difficult (Khan, 2012). In the established forest, the management of nematodes infestation is a difficult exercise, and therefore, control efforts are largely directed to forest nurseries.

The chemical approach involves the use of pesticides particularly nematicides and insecticides. Nematicides are effective against diseases caused by pine wilt nematode. For example, disulfton, Thorazine, and fensulfothione are said to be effective in managing Bursaphelenchus spp (Khan & Anwer, 2011). They are injected in tree trunks where they remain active against nematodes for two to three years upon application. The use of emamectin and

abamectin to control pine wilt disease has been scientifically established but their application is restricted because of their shorter residual effects. It requires reapplication every two years. Weevils are successfully killed through the foliar spray of carbaryl on healthy trees (Gantait, 2010; Khan, 2012). They die when they attempt to feed on the leaves or buds. Spraying the tree trunks with morantal tartrate is also recommended by nematologists but because of cost issues, its application is limited. It is only restricted to trees on the nursery (Orwa et al., 2009). Quinolizidine alkaloid, derived from Sophora alopecuroides, has been shown to be effective in the control of Pine Wilt Nematode in further studies. It has been used in some South Korean forests, where cutting down infected trees has also proven to be successful. According to research, nematode-infested trees in Korea are cut into small pieces, between one and two meters long, and treated with metham sodium before being covered with a vinyl board. In less than seven days, this approach resulted in 100 percent nematode and vector mortality. Antibiotics have also been developed, such as OA (Oxolinic Acid), which controls pine wilt disease 71% of the time (Kha & Anwer, 2011). In India, experts are deploying Thimet in seedbed to reduce the impact of nematode damage.

In the past decades, limited options for managing nematodes have been explored (Khan, 2012). The control of pine wilt nematode has been given more attention because it is the most popular. In nurseries, crop rotation and fallow method are recommended for controlling and managing parasitic nematodes in forest nurseries. The needle nematodes are controlled easily using crop rotation with a non-host as well as one year of fallow. Khan (2012) adds that in fig plantation, good sanitation practices in the nursery that seek to eliminate sources of nematode contamination can help greatly to reduce the infestation of *H. fici* nematode. In fig plantations, nematicides have

not been used because of their low economic value. Studies show that *Pratylenchus penetrans* and other nematophagous fungi are used effectively for the biological control of cyst nematodes.

The application of biological control agents has been preferred in parts because of their success and because of environmental factors. Indeed, because of environmental sustainability issues, indiscriminate use of pesticides has been questioned and alternative means of insect pest control have been recommended. Pesticide use leads to water contamination, resistance development, and killing wildlife. These demerits have favored the application of biological control methods, particularly the Entomopathogenic Nematodes (Sharma et al., 2011). Research shows that biological control methods are as lethal as chemical methods. On this note, different species of EPNs have been used to control different Plant-Parasitic nematodes. EPNs are increasingly being used because of their successful bioactivity against several economically important insect pests. They exploit insect pests such as bacteria, fungi, and nematodes effectively. They are suitable biological control for a majority of soil-dwelling stages of insect pests. They act fast and destroy the target insects in less than 48 hours. EPNs are recommended not only because they are fast-acting but also because they are safe to human and non-target organisms and the environment at large (Sharma et al., 2011). According to (Khan, 2012), applications of phosphate solubilizing microbes are fundamental in suppressing any infestation of nematodes.

For the control of timber nematodes in pine and other conifer trees, the best management strategy to control nematode infestation is to grow locally adapted pines or/and resistant pine species (Khan & Anwer, 2011). Further, eradicating the infested trees is also fundamental because it can, indirectly, influence resistance to control agents as well as impact the distribution pattern of most pine species. Further research has shown that inoculating pine trees with avirulent strains can trigger the development of systemic resistance against the virulent strains of *Bursaphelenchus*

xylophilus (Orwa et al., 2009). It has been suggested that infection of nematicides in the trunk of trees can help minimize the damage, at a small-scale level. Quarantine and fumigation with methyl bromide are effective to control strategies for the pine wilt nematodes. The ground and aerial sprays of fenitrothion have been found to be effective in reducing beetle vectors.

4.9 Studies related to nematodes impact on forestry either positive or negative

Nematodes' infestation in forests has been identified as a threat to the growth and survival of many trees in major global forests. Despite being microscopic, their impact on forests is dreadful. They are either beneficial or harmful in forestry. The root-feeding herbivores, for example, do reduce the competitive ability of plant species. Feeding on their roots weakens and limits their growth. At the same time, they indirectly promote the performance and thriving of co-existing non-host species (Khan, 2012). Evidential research shows that parasitic nematodes damage tree roots and eventually kill forests. There exist records of where these microorganisms have brought down forests in record time. *B. xylophilus* has been documented for its impacts on the forest ecosystem. Once parasitic nematodes get into a tree, they do spread rapidly occupying the tree trunk, roots, and branches. Within a few months, forest trees start wilting. In Europe, evidential reports indicate that pine wilt disease has claimed millions of pines resulting in significant ecosystem problems and the entire forestry industry.

Nematodes, as mentioned before, they do not spread on their own (Khan & Anwer, 2011). They hitch on hosts, insect hosts. In pine plantation, the hosts are pine sawyer beetles. Bacterial and fungal feeding nematodes affect plant species indirectly by feeding bacteria and fungi that facilitate the release of nutrients locked in microbes. The predation of herbivorous nematodes by carnivorous nematodes helps increase nutrient availability in the soil thus boosting the growth of

plants. (Renčo et al., 2019) analyzed the impacts of parasitic nematodes in some European forests and found that they play significant roles in the destruction of forests. They determined that forests are where nematodes are present, there is continuous degradation of trees. Khan (2012) states that nematode infestation in Congo Forests destroyed plantations of eucalyptus trees. The trend was observed in North Queensland in Australia where *Pyterygophorosoma alticolum* (Verhoef, 1984) was found to have destroyed eucalyptus trees. (Baltensweiler et al., 1988) study noted that there has been higher tree mortality in *Pinus thunbergiii* plantation located in central Honshu, Japan. The author state that nematode infection weakened trees and caused them to wilt slowly and eventually caused thousands of them to dry. In North America, severe mortality of pine trees in pine forest has been recorded in the forest where there is heavy nematode infestation.

4.9.1 EPN combined with other insect killing agents

While EPNs have been proven to be an effective biological pest control method, further research shown that its efficacy can be improved by combining it with another application. Several evidential studies have been conducted by different researchers in diverse fields to determine whether EPNs spray can be combined with chemical pesticides to boost their effectiveness in controlling and fighting insect pests. However, most of these studies have not been done on the forest but vegetable crops, wheat plantation, and fruit farms. Portman et al. (2016) did an experimental study to determine the effects of adding adjuvants to EPNs. Their study hypothesis was that adding adjuvants to sprays containing EPNs will boost their efficacy by increasing their ability to kill the target insect pest. The study revealed that adding adjuvants to EPN spray improves its effectiveness. They noted that when EPNS was combined with adjuvants, it was efficient in managing above-the-ground pests better than EPNs did alone (Portman et al., 2016).

Their experiment was done on a wheat farm where the researchers noted that the mixture was so effective in killing the diapausing wheat stem sawfly hidden in the stem.

In another experiment, Portman et al. (2016) also noted that adding Penterra, Sunspray 11N, Silwet L-77, or even Syl-Tac to EPNs solutions increased insect pest mortality by a whopping 29.1%. (Özdemir et al., 2020), in their study to find out the compatibility of EPNs with pesticides, they noted they noted similar findings as Portman et al. (2016). They noted that EPNs register high efficacy levels when combined with some select pesticides. They combined EPNs solutions with imidacloprid and cyflumetofen to control certain insects in vegetables. They found that upon mixing EPNs with said chemicals, the mortality of the insects was boosted while infectivity was significantly reduced. The findings by Portman et al. (2016) and Ozdemir et al. (2020) are consistent with earlier observations by (Rovesti & Deseö, 1990). The dual had found similar results when the tested the possibility and efficacy of combining EPNs with chemical pesticides. The combination of EPNs with other chemicals is important because EPNs survive for a few hours on the aboveground application. The rate of desiccation for EPNs is high which means that they can only be effective for a few hours upon application. However, when adjuvants or humectants are added to the solution containing EPNs, their efficiency is boosted. Therefore, adding EPNs to other chemical pesticides when it comes to the control of forest insect pest, it would be certainly effective. Having worked successfully in vegetable crops and wheat plantation, the same results can be obtained in forestry pest management.

5.0 Results and Discussion

The management of forest insect pests is fundamental because pests continue to pose significant threats to the survival of forests. Research shows that plant-parasitic nematodes have been major threats to eucalyptus, acacia trees, angiosperms, mangrove, and bamboo trees. Over the years, the use of Entomopathogenic nematodes to control parasitic nematodes in major global forests have accelerated in part because of their efficacy as well as due to environmental sustainability issues. The use of EPNs represents biological control strategies and continues to be encouraged as the most environmentally friendly control mechanism. In the past decades, there has been a massive production of EPNs for commercial use. The in vitro and in vivo methodologies have been used for this purpose. The two methods have distinct advantages and disadvantages with respect to economies of scale, production cost, technical know-how needed, and quality of the product. The in vivo method is the easiest process in terms of set up and establishment, but it is not economically feasible. The efficacy of EPNs in the forest ecosystem is affected largely by biotic and abiotic factors. Temperature, soil texture, UV light, soil aeration, soil type, carbon dioxide level, and heat are some of the abiotic factors that influence the performance of EPNs. While EPNs are quite effective on their own, their efficacy can be boosted by combining them with other chemical pesticides. Evidential research studies have revealed that when EPNs are combined with proven chemical pesticides, they increase insect mortality by a significant margin.

6.0 Conclusion

EPNs have proven to be effective and excellent biocontrol agents for plant eating insects. They have shown that they can easily achieve elimination of insect pests from forest plantation and protect trees from pest-induced wilting and withering. However, to make them even better, it is necessary to merge the right nematode species against the target pest. Further, the manufacturer should improve nematode formulation technologies to ensure that EPNs produced are able to withstand conditions posed by environmental factors such as temperature, UV radiation, soil moisture, and humidity. If these factors are not taken into consideration when producing and formulating ENPs, it is easier not to achieve the desired success. While both in vivo and in vitro methods are effective in producing EPNs, the application of in vivo methodology is limited because of the cost of labor and insect media. It is therefore an unsuitable method for commercial production.

7.0 Recommendations

Manufacturers should consider improving the technology to make it cost-friendly for both commercial and non-commercial use. In vitro methodology is cost-friendly and therefore, suitable for commercial use only. However, it can become even cheaper if insect hosts are produced in-house. In both methods, there is a need for technical improvements to expand efficiency. Improving the efficiency of producing liquid culture is necessary considering that in vitro liquid culture is the most successful when it comes to the management of parasitic nematodes. Another recommendation is the need to consider combining EPNs with other chemicals. As mentioned, EPNs efficacy rate improves when they are combined with the right chemical pesticides. In the case of forest pest management, it would be necessary to combine with certain pesticides considering the desiccation rate for EPNs is quite high. Further research is needed to come up with new strategies on how the production cost can be lowered and boost mass production and availability of EPNs. If the cost is brought down and made accessible and affordable to all people, the globe will make significant strides in sustainable environmental management.

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