Chapter

Role of Entomopathogenic Nematodes in Organic Farming and Sustainable Development

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Abstract

Chemical fertilizers and pesticides are presently accumulating in the environment harming the ecosystem, causing pollution, and spreading some of the diseases. Nematodes can be considered as entomopathogenic (EPN) if they fulfill criteria for entomo-pathogenicity when they bearing a pathogenic bacterium within a dauer juveniles juvenile, releasing the bacterium inside the host, actively seeking out and penetrating the host, rapid insect death, nematode and bacterial reproduction, reassociation of the pathogenic bacteria with new generations of dauer juveniles, and emergence of IJs from the cadaver so that the cycle can be repeated. Synthetic chemical pesticides have various disadvantages which include crop and soil contamination; killing of beneficial fauna and flora; resistance development in insects and adverse effects due to contamination in food chain; and other environment-related issues. To minimize pesticides contamination, EPN were identified as biological control agents and most suitable natural enemies of problematic insects because they reduce risk to humans and other related vertebrates.

Keywords: chemical fertilizers, entomopathogenic, pesticides, pathogenicity, biological control

1. Introduction

Around the world, growing vegetables has become a significant source of revenue for farmers. Vegetable fields make up about 7% of all croplands worldwide, and this number is typically greater in richer nations [1]. Vegetable fields differ from crop fields in that they apply more nitrogen, produce their crops more intensively, use more tillage and irrigation, and have more planting-harvest cycles throughout the year [2]. For instance, fertilizer inputs in vegetable cultivation reached 600 kg of nitrogen per hectare per year [3], compared to 300 kg of nitrogen per hectare per year in cereal cropping systems [4]. It has been demonstrated that intensive farming decreases soil biodiversity, which is crucial for the health of ecosystems [5, 6]. Therefore, it is critical to look into potential impacts of intensive agricultural methods on soil nematode communities. According to [7, 8], organically managed farmlands have been increasing to about 107 ha globally and are anticipated to continue growing. Organic farming systems are generally thought to be more sustainable than conventional farming systems [9]. In India, organic vegetable growing is likewise showing an upward trend. According to two global meta-analyses, organic farming benefits the soil biota [10]; however, a thorough understanding of whether and how organic management affects the community structure of soil nematodes and related functions is currently lacking. Although the effects of organic farming on soil nematodes have been evaluated in grasslands [11], arable fields [12]; orchards [13]; and vegetable fields [14]. Organic farming may have a negative impact on soil nematodes. Change depends on the crop type, soil texture, and past land use [15]. Because nematode abundance and community composition can be related to edaphic and climatic fluctuations at different scales, the impacts of organic farming on soil nematode community may depend on spatial scale [16]. Finally, the comparative impact of organic farming on soil nematode communities across various vegetable types is largely unknown because previous studies assessing the effect of organic farming on soil nematodes frequently focused on single vegetable types such as tomato [14], green peppers [17], and asparagus [18].

Insect-parasitic nematodes, known as entomopathogenic nematodes (EPNs), have been described from 23 nematode families [19]. Among all nematodes that have been studied for insect biocontrol, Steinernematidae, and Heterohabdichidae have received the most attention because they possess many properties of effective biocontrol agents [19]. It has been used as a classical antiseptic and as an adjunctive biological control agent. Most of the applied research has focused on its potential as an adjunctive biological control agent applied to floods [20]. Extensive research over the past three decades has demonstrated both successes and failures in pest control of crops, ornamental plants, lawns, and peat [21, 22].

Extensive studies have demonstrated both success and failure in controlling pests on crops, trees, ornamental plants, lawns, and peat [23]. EPNs occur naturally in soil and are divided into the Steinernematidae and Heterorhabditidae families [22]. EPNs form a persistent or stress-tolerant stage known as infectious juvenile (IJ) [24]. This developmentally retarded stage also plays an important role in nematode dispersal in soil, as nematodes actively seek out and infect suitable insect hosts [25]. In addition, IJs play a role in transferring entomopathogenic bacteria from one host to another. After localizing and invading the insect host, IJs migrate to the hemolymph, where they recover from their arrested developmental state and release their bacterial symbionts. The bacteria multiply, release toxins, and kill the insect within 24–72 hours. EPNs traverse the soil by following chemical signals (chemotaxis). Through chemotaxis, they recognize hosts in their environment or areas where hosts are likely [26]. Several studies have shown that migration of EPNs in soil is also affected by other factors such as CO_2, plant exudates, pH, temperature, electrical potential, and VOCs [27]. The use of EPNs in biological control has traditionally been associated with the control of soil-dwelling pests [23]. Studies over the past two decades indicate that they may also control airborne pests, but only under certain circumstances [28]. The reduced effectiveness of EPNs in controlling aerial pests is primarily due to exposure to ultraviolet light [29], temperature extremes [30], and inadequate humidity [31]. These factors are important for EPN survival. For this reason, outdoor EPNs are less efficient against airborne pests, although previous laboratory tests have shown much higher efficiencies [32]. The most common EPN formulation is an aqueous suspension [33]. Equipment intended for the application of pesticides, fertilizers, or irrigation can be used for EPN applications. Backpack, hand or tractor sprayers, and sprinklers are suitable for this. IJs can pass through spray tubes of at least 500 μm diameters and withstand pressures up to 2000 kPa [34]. In addition, IJ withstands short-term exposure (2–24 hours) to many chemical and biological pesticides, fungicides, herbicides, fertilizers, and growth regulators and can be mixed in a tank and treated as such. It can be applied together with the product [35]. The combination of nematodes and insecticides in tank mixes can provide a cost-effective alternative to foliar-integrated pest management (IPM) systems. Because nematodes are sensitive to UV light, nematodes should be applied to plants in the evening, early morning, or during cloudy weather when the radiation is less intense [36]. Nematode survival and foliar efficacy are improved to varying degrees by adding various adjuvant with anti-drying (e.g., glycerol and various polymers) or UV-protective (brightener) effects to the spray mixture [37], but much more needs to be done to improve survival after application. Arguably, the greatest potential for the use of EPNs against foliar pests is in combination with other biological control agents [38] or selective chemicals [39]. In the IPM program, EPNs are considered particularly safe biological agents [40]. Due to their specific effects, they pose no environmental risks other than chemical pesticides [41]. Since EPN was first used in the United States in his 1841 to control the *Popyria japonica* Newman beetle, no cases of environmental damage by EPN have been documented [42]. Using nematodes is safe for users. EPN and its associated bacteria do not harm animals or plants [43].

1.1 Impact of chemical pesticides

1.1.1 Impact on environment

Chemical pesticides can infect soil, water, lawn, and other undergrowth. Insecticides not only kill insects and weeds, but can also be toxic to a wide variety of organisms, including birds, fish, beneficial insects, and unwanted plants. Insecticides are generally the most toxic class of pesticides, but herbicides can also pose risks to non-target organisms.

1.1.2 Impact on humans

Application pesticideswill kill the disease causing insects thereby increasing the food and fiber production. There is now irresistible evidence that some of these chemicals pose potential risks to humans and other living organisms and have adverse effects on the environment [44]. No population is completely resistant from exposure to pesticides, and potentially severe health effects are disproportionately borne by people in developing countries and high-risk groups in each country [45]. Worldwide, pesticide poisoning causes approximately 1 million deaths and chronic illnesses annually [46]. Groups at high risk of exposure to pesticides include production workers, formulators, sprayers, mixers, shippers, and farm workers. The processes involved are not without risk, and the potential for hazards during manufacturing and formulation can increase. Industrial environments pose increased risks to workers as they work with a wide variety of toxic chemicals, including pesticides, raw materials, toxic solvents, and inert carriers.

Organochlorine pesticides can infect the tissues of virtually all life forms on Earth, the air, lakes and seas, the fishes that live in them, and the birds that feed on them [47]. The National Academy of Sciences in United States found that the DDT metabolite DDE caused eggshell thinning and that the bald eagle population declined primarily due to exposure to DDT and its metabolites [48]. Certain environmental chemicals, including pesticides, called endocrine disruptors, are known to produce adverse effects by mimicking or antagonizing natural hormones in the human body, and their long-term low-dose exposure may have implications for human health, including immune suppression, hormone disruption, decreased intelligence, abnormalities in reproduction, and cancer etc. [49].

1.1.3 Impact on food materials

A program entitled "Monitoring of Pesticide Residues in Products of Plant Origin in the European Union" has been set up in the European Union since 1996 to determine the extent of pesticide residues in food. In 1996, seven pesticides (acephate, clopyrifos, clopyrifos-methyl, methamidophos, iprodione, procymidone, and chlorothalonil) and two groups (benomyl group and maneb group, *i.e.,* dithiocarbamates) were tested to apples, tomatoes, lettuce, strawberries, and grapes [50].

In India, the first report of pesticide poisoning was in Kerala in 1958, where the pesticide caused mortality more than 100 people from eating wheat flour contaminated with parathion [51]. This led to the dedication of the special committee set up by ICAR on the Harmful Effects of Pesticides (ICAR Task Force Report, 1972). In an interdisciplinary study evaluating pesticide residues in selected foods collected in different states of the country (Surveillance of Food Contaminants in India, 1993), DDT residue was detected approximately 82% of 2205 samples of milk collected from 12 states. Approximately 37% of the samples contained DDT residues above the acceptable limit of 0.05 mg/kg (whole milk basis). The maximum amount of DDT residue detected was 2.2 mg/kg. The proportion of samples containing residues above acceptable limits was highest in Maharashtra (74%), followed by Gujarat (70%), Andhra Pradesh (57%), Himachal Pradesh (56%), and Punjab (51%). In other federal states, this percentage was less than 10%. Data from 186 samples from 20 brands of infant formula showed that approximately 70 and 94% of the samples had DDT and HCH isomers at maximum levels of 4.3 and 5.7 mg/kg (fat basis), respectively. Measuring chemicals in the entire diet provides the best estimate of human exposure and potential risk. Risk to consumers can be assessed relative to toxicologically acceptable intake levels. The average total intake of DDT and BHC by adults was 19.24 and 77.15 mg/day, respectively [52]. Fatty foods were the main source of these contaminants. Another study reported that her average daily intake of HCH and DDT by an Indian was 115 and 48 mg per person, respectively, which is the amount observed in most developed countries [53].

1.1.4 Impact on soil

Numerous transformation products (TPs) from a wide range of pesticides have been documented [54]. Pesticides and TPs can be classified as follows: (i) Pesticides that exhibit such behavior include organochlorine DDT, endosulfan, endrin, heptachlor, lindane, and their TPs. Most of them are now banned from agriculture, but their residues still exist. (ii) Polar insecticides are represented mainly by herbicides, but also carbamates, fungicides, Phosphorus insecticide TP are also included. They can be moved from soil by runoff and leaching, thereby constituting a problem for the supply of drinking water to the population. Herbicide pesticide TPs are unquestionably the most studied pesticide TPs in soil. Numerous metabolic pathways that involve transformation through hydrolysis, methylation, and ring cleavage and result in the production of several toxic phenolic compounds. Pesticides and their TPs are retained in soil to varying degrees, depending on the interaction of soil and pesticide properties. The most influential soil property is organic matter content. The higher the organic matter content, the greater the adsorption of pesticides and TPs. The soil's ability to retain positively charged ions in exchangeable form is important for paraquat and other positively charged pesticides. Strong mineral acids are required to extract these chemicals, but no analytical improvements or studies have been reported in recent years. Soil pH is also important. Adsorption increases with decreasing soil pH for ionizable pesticides (2,4-D, 2,4,5-T, picloram, atrazine, etc.) [55].

1.1.5 Impact on soil fertility

Severe treatment of soil with pesticides can reduce the number of beneficial soil microorganisms. Soil scientist Dr. Elaine Ingham said "Overuse of chemical fertilizers and pesticides has similar effects on soil organisms as overuse of antibiotics by humans. The indiscriminate use of chemicals may be effective for a few years, but after a while there will not be enough beneficial soil organisms to hold the nutrients" [56]. Mycorrhizal fungi grow with the roots of many plants and aid in nutrient absorption. These fungi can also be damaged by herbicides in the soil. One study found that both oryzalin and trifluralin inhibited the growth of certain types of mycorrhizal fungi [57]. Roundup has been shown to be toxic to mycorrhizal fungi in laboratory studies, with some adverse effects observed at concentrations lower than those found in soil after normal application [58]. Triclopyr was also found to be toxic to some mycorrhizal fungi [58], and oxadiazon decreased mycorrhizal fungal spores numbers [59].

1.1.6 Impact on surface and groundwater

Pesticides can enter surface waters through runoff from treated crops and soil. Pesticide infection of water is widespread. Results of a comprehensive series of studies conducted by the United States Geological surveys (USGS) conducted in major river basins across the country have returned surprising results. More than 90% of water and fish samples from all rivers contained one or more pesticides [60]. Pesticides were detected in all major river samples with mixed agricultural and urban land use impacts and in 99% of urban stream samples [61]. According to the USGS, more pesticides were found in municipal than agricultural waterways [62]. The herbicides 2,4-D, diuron, and prometon, and the insecticides chlorpyrifos and diazinon, all commonly used by urban homeowners and school districts, are among the most common in surface and groundwater sources nationwide. Was among the 21 pesticides found in the United States [63]. Trifluralin and 2,4-D were detected in water samples from 19 of the 20 river basins studied [64]. The USGS also found that pesticide levels in urban waterways often exceed guidelines for protecting aquatic life [65].

Groundwater contamination by pesticides is a global problem. According to the USGS, at least 143 different pesticides and 21 transformation products have been detected in groundwater, including pesticides from all major chemical classes. Evidence has been found in groundwater in more than 43 countries in the last 20 years [66]. A study in India found that 58% of drinking water samples collected from various hand pumps and wells around Bhopal were contaminated with organochlorine pesticides exceeding EPA standards [67]. When groundwater becomes contaminated with toxic chemicals, it can take years for the contaminants to dissipate or be cleaned up. Cleaning can be very expensive and complicated, if not impossible [66].

1.1.7 Impact on non-target fauna and flora

Pesticides are common pollutants found in non-target organisms in soil, air, water, and urban landscapes. Once there, it can cause plant and animal damage, ranging from beneficial soil microbes and insects to non-target plants, fish, birds and other wildlife. Drift occurs, even from terrestrial instruments [68]. Drift can represent a loss of 2–25% of the applied chemical and can extend over distances of a few meters to hundreds of kilometers. Up to 80–90% of applied pesticides can volatilize within days of application [69]. Research on this topic is limited, but research continues to find pesticide residues in the air. According to the USGS, airborne pesticides were detected in all US sample areas [56]. Herbicides are designed to kill plants, so it is not surprising that direct application to such plants, or drifting or volatilizing, can harm or kill desirable species. Formulated herbicides have been shown to volatilize untreated plants with enough vapors to cause severe damage to other plants [70]. In addition to outright killing non-target plants, exposure to insecticides can have sublethal effects on plants [71]. Exposure to the herbicide glyphosate can severely affect seed quality [72]. It may also increase susceptibility to certain plant disease. This poses a particular threat to endangered plant species.

Entomopathogenic nematodes (EPNs) are members of the soil biota and provide biological control of arthropod pests, an important ecosystem service in agriculture. Their infective juvenile (IJ) stage occurs naturally in soils where arthropods can coexist and serve as hosts, ranging from marine areas to alpine areas, natural to agroecosystems, and even heavily polluted. It is distributed in soils that have been extensively treated [73]. Research on EPNs in the context of agroecology and applied soil ecology has increased in recent decades [74]. Several studies have demonstrated how changes in soil properties affect EPN communities and related organisms such as nematophagous fungi (NFs) and free-living nematodes (FLNs), their natural enemies, and potential competitor [75]. EPNs are considered particularly safe biological agents [40]. Due to their specific effects, they pose no environmental risks other than chemical pesticides [41]. Since EPN was first used to control the *Popylia japonica* Newman beetle in the United States in 1841, no cases of environmental damage from EPNs have been documented [42]. Using nematodes is safe for users. EPN and its symbiont bacteria are harmless to mammals and plants [43].

1.2 Identification of entomopathogenic nematodes

The first species of entomopathogenic nematodes was described morphologically in 1923. Adults of first and second generations and third stage IJs of *Steinernema* and *Heterorhabditis* possess some distinctive morphological features which are very important from the taxonomic point of view. However, it was become a monotonous task to categorize the increasing number of species with these taxonomic characteristics. Therefore, certain ratios and De Man Indices were created in order to delineate the species more appropriately. These ratios are based on the following characteristics, *viz*. tail length; position of excretory pore, nerve ring, and pharynx length. Besides these, males acquire some prominent characters such that spicule and gubernaculums. Analysis and measurement of these traits are playing a key role for identifying species. The vulva, which is a well-known feature of the females of entomopathogenic nematodes, its position, and associated structure are also important traits, provided by taxonomists with a clear method for identifying the species. The SEM investigations of first-generation males revealed the complete structure of gubernaculums and spicules, the presence or absence of caudal mucron, the position of the copulatory papillae, the morphology of spermatozoons [76], and the presence or absence of small cuticular projections, or epiptygmata, which protect the opening of the female vagina [77]. Head contour, cephalic horns, the lateral field, tail length and shape, and so forth are some of the crucial traits of taxonomic significance for IJs [78].

Due to the increase in the number of species, morphological characterization no longer provides accurate results and makes molecular characterization essential for species identification. Morphology is completely determined by the external characteristics of the specimen; however, some genes tend not to reveal themselves in phenotypes despite it having conserved portions that are crucial from a taxonomic perspective. In addition, morphology is a laborious task that needs for qualified taxonomists with the necessary knowledge. In order to confirm the taxonomic position of a certain species and its validity, this creates a necessity for molecular identification and characterization. Advancements in the molecular techniques help in the precise identification and placement of the species in its appropriate position in the classification. Moreover, the phylogenetic relationships of the species with the other species of a genus and with other orders are also established by utilizing the modern and advanced tools of molecular characterization. A number of molecular techniques are being used for more precise identification of EPNs as immunological techniques [79]; isoenzyme patterns [80]; total protein patterns [81]; and RFLP detection within total genomic DNA [82].

1.3 Life cycle of entomopathogenic nematodes

Steinernema and *Heterorhabditis* have comparable life cycles. Between a free-living stage and a parasitic stage, both genera maintain stability. An outermost cuticle acts like a barrier between the environment and the free-living form of EPNs. The invasive EPN stage, also known as the infective juvenile (IJ) or J3 stage, is encapsulated and unable to feed since its mouth and anus are sealed [83]. To be capable of surviving without a host for several months, they actually have enormous lipid storage [84]. It has been observed that IJs of *Steinernema* live much longer in the environment than IJs of *Heterorhabditis*, while having similar lipid reserves, it may be explained by the IJs' motile behavior. According to findings, Heterorhabditis IJs nictate between 70 and 90% of their lives, compared to *Steinernema* IJs' 50 to 80% [85], and as a result, lipid reserves are depleted more quickly in *Heterorhabditis* IJs. Infective juveniles wait for insect larvae up to 20 cm deep in soil [77]. In case of *Steinernema*, IJs invade the insect larvae through natural openings such as the mouth, anus, spiracles, and wounds [86]. However, in case of *Heterorhabditis,* the IJs are also able to penetrate the insect body by directly scratching their cuticle because of the presence of a large anterior tooth [87]. IJs lose their cuticle and release the entomopathogenic bacteria (EPB) after fettling entering in the insect body. Together, they eventually kill the insect. On usually, 3 days after an insect infestation, IJs begin feeding on the insect cadaver and eventually reach the fourth stage juvenile (J4), which divides into males and females. First generation (G1) females lay eggs after mating, either in the external medium or still inside the female's body and these eggs hatch into first-stage juveniles (J1). Depending on the quantity of food is left in the insect cadaver at that point, types of situations are possible. In the case of insufficient food, J1 immediately transformed into the second-stage juvenile (J2) in 2 or 3 days. Before becoming an infective juvenile, J2 stopped feeding and had a molt while still in the pre-infective stage. Then the newly generated IJ emerge from the depleted insect cadaver to actively look for another susceptible insect prey. On the other hand, if there is an abundance of food in the cadaver, both males and females can reproduce numerous generations in the same cadaver. After hatching from the eggs of the G1 females, J1 turns into J2, non-infectious J3, and J4 before becoming the second generation (G2) of adults. After mating, G2 females release eggs that develop into J1s, starting a new cycle. EPNs typically reproduced for two to three generations before the food sources in the insect cadaver are completely depleted, [86]. After insect invasion by IJs, the entire reproductive cycle lasts 7 to 14 days and is mostly dependent on temperature. After mating with male, both *Steinernema* and *Heterorhabditis* females lay eggs in the dead insects cadaver. Eggs that have been hatched usually develop juveniles that grow and develop to be amphimictic adults [88]. The reproductive life cycle of the majority of *Steinernema* has sexually distinct partners, G1 males and females, while all *Heterorhabditis* IJs after insect infection become self-fertilizing hermaphrodite females. However, amphimictic *Heterorhabditis* adults are developed by the second generation. It is interesting to note that IJs from the *S. hermaphroditum* species can mature into self-fertilizing hermaphrodite females, rather like IJs from *Heterorhabditis* do. The unusual characteristic of this *Steinernema* species has been proposed to support the independent but converging evolution with *Heterorhabditis* described by Poinar and previously described [89]. *Heterorhabditis* EPNs reproduce hermaphroditically, which greatly reduces or impairs the genetic diversity of the offspring. *Heterorhabditis's* hermaphrodite behavior makes it possible for a single IJ to infect a host and molt into a hermaphrodite female [90], whereas at least two *Steinernema* IJs must penetrate an insect larva and reach maturity into male and female to cause infection. This undoubtedly represents *Heterorhabditis* species a survival edge over *Steinernema* species. The process of fertilizing the female's eggs with sperm occurs during male and female mating. Male releases spermatozoids into the female's vulva along with its spicule, which it uses to develop spermatozoids. In the uterus, the female's eggs are fertilized by the male's sperm. For hermaphrodites female who are self-fertile, sperm is formed and stored in spermatic vesicles, which are described as a distal enlargement of the uterus. When the female initiates to lay eggs, the sperm in the spermatic vesicles automatically fertilizes them. Since females are longer and bigger than males, males need to find a strategy of scanning the full female body in order to find the vulva. The two ways that a male identified the vulva on a female body. These two reproductive strategies highlight still another difference between *Steinernema* and *Heterorhabditis*, namely that males stick to females and slither all over their bodies until it finds the vulva, but both the female and male heads of *Heterorhabditis* point in the opposite directions. [91]. According to *Steinernema*, the males behave like a ring around the female body. Until reaching the vulva, the male coils entirely around and along the female body [26]. There are some safeguards that have been adopted, to prevent multiple males from mating with the same female. In *Heterorhabditis* species, after mating, a male releases a mating plug that closes the vulva, preventing other males from mating with the same female [92]. In *Steinernema* species, it has been proved that virgin females generate various chemicals that attract males and that their production reduces off after mating [26]. Moreover, male of *S. longicaudum* required virgin conspecific females to mature in their immediate surroundings [93].

1.4 Mode of action

After mating, most of the eggs are preserved inside the maternal body of the EPN. Following that, the offspring grow and feed inside the maternal body. This process is called endotokia matricida which is derived from the Greek word ενδο ("endo", inside) and τοκοσ ("tocos", birth), and from the Latin ("mater", mother and "caedere", kill). This term was coined by Maupas, (2015) when he first characterized the *Caenorhabditis elegans*. This phenomenon helps the progeny by protecting it and, in the case of EPNs, by giving it a high-lipid food source, particularly whenever the infected insect cadaver is about to be exhausted. When endotokia matricida is encouraged in cases where there is a lack of food, this phenomenon takes place to the first generation of juveniles even when there is still a plenty of food. It follows that it is clear that the size of the vulnerable insect will have an impact on the growth and survival of EPNs. According to some authors, *Steinernema* IJs are ineffective at controlling micro-insect pests [94]. The infectivity of four different *Steinernema* species in insects smaller than 5 mm in length was recently demonstrated by Bastidas and coworkers [95], who also came to the conclusion that *Steinernema* and *Heterorhabditis* nematodes cannot survive in the environment for very long if there are no larger insects available for them to complete their life cycle.

2. Biological control

Biological control is an eco-friendly and effective means of reducing or mitigating pests through the use of natural enemies [96]. It relies on predation, parasitism, herbivores, or other natural mechanisms, but involves an active human management role [97]. According to Dreistadt (2007) "*Biological control is the beneficial action of predators, parasites, pathogens, and competitors in controlling pests and their damage*". Biological control now becomes an interdisciplinary science combining entomology, microbiology, plant pathology, weed science, and virology with the goal to reduce and control pathogens, microorganisms, insects, and plants alike, which can cause damage to crop plants [98]. The different biological control agents have been used time to time, and their success and failures have been extensively reviewed. Their use in bio-control has increased over recent decades.

Classical biological control involves usage of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control [99]. Classical biological control focuses on finding natural enemies, introduces them into the area of the target pest, and permanently establishes them so that they will provide continuing pest control with little or no additional human intervention. Augmentation on the other hand involves deliberate discharge of natural agent that does not occur in good numbers and thus are incapable of reducing pest below damaging level [100]. There are two general approaches to augmentation: inundative releases and inoculative releases. The former involves usage of large number of natural enemies for immediate pest control by disseminating them on the crops fields multiple times, while later method involves release of small natural enemies at given intervals in order to keep pest populations below economic injury level. The last type, conservation biological control involves measures of modifying existing practices to further enhance specific natural enemies of other organisms to reduce the effect of pests.

Biological control agents include bacteria, fungi, viruses, nematodes, or protozoa that can infect and kill the host. Some of these agents can kill and infect insects and are referred to as entomopathogens. Among entomopathogens, there is class of nematodes which parasitize insects only and are referred to as entomopathogenic nematodes (EPN) which are associated with entomopathogenic bacteria. The entomopathogenic nematodes, belonging to the families, Steinernematidae [101] and Heterorhabditidae [102], are associated with bacteria which belong to the family Enterobacteriaceae [103], *Xenorhabdus* [104] in case of steinernematids and *Photorhabdus* [105] in case of *Heterorhabditids* which reside in their alimentary canal. At present two well-known genera, *Steinernema* and *Heterorhabditis* of EPNs, are globally described and consisting more than 100 species and 21 species, respectively [106]. They all are lethal duo, capable of killing the host within short duration and hence are considered as good bio-control agents [107]. The bacteria mostly resides in the alimentary canal of third stage juveniles, called infective juveniles (IJ) which is only free living stage in the life cycle of EPN. They live freely in moist soils and move in search of their insects hosts. Once, they come in contact with the insect host, they enter in their body either through natural openings or by abrading the skin. After their entry in the insect host, they release their endosymbiont bacteria and kill the host within 24 to 48 hours. They feed on the insect cadaver and produce their adult generations, which mate inside the cadaver and released the eggs which hatched into juvenile stages. Once the survival resources are depleted, they move out the cadaver and search for the next host.

2.1 Entomopathogenic nematodes in insect pest management

Researchers focused on this area when *Steinernema glaseri*, the first EPN species introduced as a biocontrol agent, was used in the United States against the Japanese beetle, *Popillia japonica* [108]. These organisms reemerged as effective biocontrol agents in the 1960s and 1970s, with *Steinernema carpocapsae* (also known as *Neoaplectana carpocapsae*) serving as the primary biocontrol agent. [109]. With the advancement of fermentation technology, several species of EPN (including *S. carpocapsae, S. scapterisci, S. feltiae, S. glaseri,* and *Heterorhabditis megidis*) have been mass produced commercially and are sold in market for the use by growers in formulations suitable for short term storage [110]. The mass production of IJs of EPNs is easy and cost effective. The preferred method of application is inundative release [111].

S. carpocapsae, S. feltiae, S. kraussei, S. glaseri, S. riobrave, Heterorhabditis bacteriophora, and *H. megidis* are some of the well-known most frequently used and successfully deployed nematodes as biopesticides. This characteristic is attributed to their simple and easy mass production in liquid culture [112]. Cultivation using live insect hosts (*in vivo*) requires cheap start-up costs, low levels of technology, and high nematode quality but cost-effective efficiency. *In vitro* solid or liquid culture is a cost-effective method, with liquid culture requiring the largest start-up capital.

2.2 Bio-formulations using entomopathogenic nematodes

One of the traditional ways to prevent losses from insect pests has been to use chemicals; although, nowadays days, due to several unjustified side effects, pest control relies on many other solutions in addition to pesticides. The term "Integrated Pest Management" refers to the combination of all these options (IPM). IPM is a pest management technique based on a systems approach that considers the entire ecosystem of the orchard. Continuous use of hazardous chemicals, at high doses against agricultural key pests, has led to major problems such as pest resurgence resulting from development of resistance and destruction of natural enemies. Also, the enormous use of pesticides is not only costly affair but also due to its residual effects, is directly or indirectly harmful to animals, other non-targeted soil fauna and human beings too. In search of new avenues in biological control, the importance of EPN has been highlighted as an environment-friendly pest control method. The successful market introduction of an EPN-based product requires a reliable species-specific isolate and stable formulation having more than 6 months of its shelf-life period when stored at room temperature (20–25°C) such formulations are on high demand. Unfortunately, no species-specific nematode formulation has been developed so far which could achieve the goal.

EPN formulation is a process of the transformation of living entities into a product that can be applied by practical methods. Few factors affect their application part in field conditions which include market value; crop and target insects; formulation type and shelf life; usage directions; technical support; cost and others [113]. Generally, EPN formulation contains an active ingredient, a carrier and additives. There are different types of EPN formulations present in market which are synthetic sponges [114], gels [115], clay and powder [116], or infected cadavers form [117]. Few factors like soil moisture; soil texture; water content; temperature; and UV radiation may impact drastically affect the infectivity of EPNs, storage and formulation and development of industrial product. To increase the strategies for optimization of effectiveness, timing of application and type of formulations gives best results [118]. Several formulations which have been used before include clay; polyether polyurethane sponge; anhydrobiotic nematodes; bait and activated charcoal [119].

Good storage and formulation strategies can be developed in order to ensure nematode survival and maintenance of increased infectivity [119]. This is why it is crucial to have an understanding of nematode ecology and also analyze which environmental factors affect their activity and infectivity [120]. EPN application rates vary widely, ranging between 7400 and 1,500,000 IJs/m^2 [121], but 250,000 IJs/m^2 being a common recommended rate for commercial applications [122]. Currently, S. carpocapsae, S. feltiae, S. kraussei, S. glaseri, S. riobrave, H. bacteriophora (CAB Reviews 2018 13, No. 058) http://www.cabi.org/cabreviews and Heterorhabditis megidis are the most commonly used and successfully applied nematodes due to their easily production in liquid culture [112] (**Table 1**).

In India, various studies have been conducted to improve formulations in terms of storage, shelf life, application techniques, virulence control, etc. *Heterorhabditis indica, Steinernema abbasi, S. bicornutum, S. Carpocapsae,* and *S. riobrave* are contained in a variety of carrier materials such as talc, alginate capsules, wheat bran pellets, sodium alginate beads, vermiculite, spray vehicles or hydrogels (Hussaini et al. [123, 124]; Gupta [125]; Vyas et al. [126]). However, it is currently only in the comprehensive research stage. National Institute of Plant Health Management (Hyderabad), Indian Agricultural Research Institute (New Delhi), National Agricultural and Insect Resources Board (Bangalore), Multiplex Biotech Pvt. Ltd., Ajay Biotech (India) Ltd., and Pest Control (India) Pvt. Ltd. conduct research to provide EPN formulated products with improved shelf life (**Tables 1** and **2**).

S. No.	EPN species	Product Name	Country
1.	*S. carpocapsae*	ORTHO Biosafe	USA
2.	*S. carpocapsae*	Biovector	USA
3.	*S. carpocapsae*	Exhibit	USA
4.	*S. carpocapsae*	XGNAT	USA
5.	*S. carpocapsae*	Helix	Germany
6.	*S. carpocapsae*	Boden Niitzlinge	Switzerland
7.	*S. carpocapsae*	Sanoplant	USA
8.	*S. carpocapsae*	Proactant	USA
9.	*S. carpocapsae*	Green Commandos-Ecomax	India*
10.	*S. feltiae*	Manget	USA

S. No.	EPN species	Product Name	Country
11.	*S. feltiae*	Entonem	USA
12.	*S. feltiae*	Nemasys	USA
13.	*S. feltiae*	Stealth	UK
14.	*S. riobrave*	Vector MG	USA
15.	*S. kushidai*	SDS Biotech	Japan
16.	*H. bacteriophora*	Otinem	USA
17.	*H. bacteriophora*	E-nema	Germany
18.	*H. bacteriophora*	Soil Commandos-Ecomax	India*

**Product launched in 2011 but withdrawn from the market due to inconsistency of results in the field.*

Table 1.
Worldwide used entomopathogenic nematodes-based formulations.

S. No.	EPN species	Production/ formulation	Reference
1.	*S. bicornutum*	Bait as alginate capsule	[123]
2.	*S. carpocapsae*	Alginate capsule	[123]
3.	*S. carpocapsae*	Wheat bran pellets	[123]
4.	*S. riobrave*	Spray-adjuvants	[126]
5.	*Heterorhabditis indica*	Talc	[124]
6.	*S. abbasi*	Talc	[124]
7.	*S. carpocapsae*	Talc	[124]
8.	*S. carpocapsae*	Pearl (sod. alginate)	[125]
9.	*S. carpocapsae*	Vermiculite	[124]
10.	*Steinernema .f abbasi = (Symbiobacterium thermophilum)*	Hydrogel	[127, 128]

Table 2.
Entomopathogenic nematodes-based product used in India.

3. Various formulations and their applications

In comparison with foliar pests, the performance of EPN has shown more success in controlling soil-born insect pests. Juveniles' intolerance to fluctuations of desiccation [31], temperature [30], and UV radiation [129] is a crucial factor in the failure of foliar applications of EPN Schroer and Ehlers (2005) recently used *S. carpocapsae* on cabbage to attack the foliar insect *Plutella xylostella*, which was mixed with the surfactant Rimulgan and the polymer xanthan to create the best circumstances for nematode infection on the plant surface. As an integrated pest control tool, EPN effectiveness has also been proven to be compatible with chemical insecticides, fungicides, and acaricides [130]. As a result, these chemicals can frequently be tank mixed and applied with other pesticides. Some insecticides, including Imidacloprid [131], tefluthrin [132], neonicotinoid [133] and *Bacillus thuringiensis* [134] were found to be synergistic with EPN. Hence, before EPN is released into the field, its formulation and integration with pesticides and surfactants should be carefully evaluated. Nematode control is regarded as more crucial due to the lack of possible nematicides, the high cost of field application, and the current trend toward eco-friendly pest and disease control methods.

Nematicides should not be used in the current era of globalization. Nematicides will probably no longer be available because they are expressly prohibited in organic farming. Root-knot nematode, which causes serious harm to various crops, affects a huge range of vegetables, fruits, and pulse crops in India. Since EPN is already well-established for the control of insect pests, applying it to the management of plant parasitic nematodes would be extremely cost-effective (PPN). There are various reports that indicate EPN was used to reduce PPN [135]. The impacts of the microorganisms *Xenorhabdus* spp. and *Photorhabdus* spp. that are linked to *Steinernema* and *Heterorhabiditis*, respectively, have suppressed selected species of PPN including root-knot nematode in green house experiments [23]. However, the existing literature has limited information on at what stage(s) of PPN are affected by EPN applications. Tests conducted in the field have demonstrated PPN population suppression for up to 8 weeks after application of EPN products [135].

Extensive research over the past decade has produced numerous effective isolates and strains as well as substantial advancements in mass production and formulation technology, all inspired by the need to reduce pesticide consumption [136]. Currently, *S. carpocapsae, S. feltiae, S. kraussei, S. glaseri, S. riobrave, H. bacteriophora* (CAB Reviews 2018 13, No. 058) http://www.cabi.org/cabreviews, and *H. megidis* are the most commonly used and successfully applied nematodes due to their easily production in liquid culture [112]. Today, EPN are mostly used in soil, galleries of boring insects, circumstances where insecticide resistance has occurred, or when dangerous pesticides are prohibited, conditions where chemical pesticides have failed [40].

In India, several scientists have used EPN in both lab and field conditions to attack cutworms, ragi pink borer, stem borer, white grubs, etc. [137]. Few EPNs, including *S. corpocapsae* (strain DD-136), *H. bacteriophora* (strain Burliar), and *Heterorhabditis* species, were found in field trails against *Amsacta albistrigata* larvae in their fourth instar on ground nuts [138]. Green Commandos (*S. corpocapsae*) and Soil Commandos (*H. bacteriophora*), two EPN-based formulations formed by Ecomax Compant in 1980 using exotic species, were both removed from the marketplace due to their lack of effectiveness against insect pests due to their poor adaptability to Indian environmental conditions or formulation issues.

4. Factors affecting survival and efficacy

Matching the optimal EPN species or strain to the target host and environment requires consideration of innate efficacy and suitability of environmental conditions. EPN population persistence is determined by the permanence of individual IJs and population recycling in host insect larvae and many factors that can influence both mechanisms [139, 140]. IJs in different EPN species vary in natural longevity from several months to over a year. Losses of 50% can be reached within hours after soil application until the IJ settles in the soil. Losses then range from 5 to 10% per day for 1 to 6 weeks and often only about 1% of the original inoculum survives. To compensate for these losses, a general rule of thumb for application rates is 25 IJ per cm2 of treated area, although higher or (rarely) lower application rates may be used depending on the pests targeted and the cropping system may be required. As a result, IJ populations in soil or similar substrates generally remain tall enough to provide effective control for 2–8 weeks. Post-application recycling is frequent, but not sufficient to achieve multi-seasonal control, as the distribution of IJs usually becomes too patchy over time. UV light can inactivate and kill his IJs within minutes of application, but the effects vary by his EPN strain and species [129]. If application is in the early morning or evening, IJ losses are minimized by adding a UV protectant to the IJ suspension and applying the soil with a large amount of carrier combined with immediate rinsing with sufficient water. Most EPN species perform optimally at 20–30°C [30], become sluggish below 10–15°C, and are inactivated above 30–40°C. Various *Steinernema* spp. have been isolated from other EPN species in cold, hot semi-arid, and even arid regions and may have promise for use in extreme environments. In soil, IJ travels in a film of water that covers crevices. Adequate substrate moisture is essential for good IJ activity. Dry conditions limited IJ activity, but gradual dehydration can cause IJs to enter and persist dormancy. Drought-intolerant *H. bacteriophora* IJ actively seek soil layers with high water content, whereas drought-tolerant *S. carpocapsae* IJ can survive better in drier conditions. In water logged soils, anoxic conditions and low surface tension can compromise IJ movement and even survival. Movement and survival of IJs are generally more restricted in microstructured soils than in sandy soils [141], although sandy soils may dry out faster and reduce IJ activity. EPNs are adversely affected by pH values <4 and >8. Various biological factors can also affect the survival of IJ or EPN populations in soil. Many species of arthropods and other invertebrates prey on IJs (e.g., mites, springtails, tardigrades, predatory nematodes, nematophagous fungi), or feed on EPN-infected hosts. Other entomopathogens (e.g., entomopathogenic fungi, bacteria, or viruses) or parasites compete with host EPNs [140].

5. Conclusion

The inordinate use of chemical pesticides in farming causes serious damage to soil, air, water, flora, fauna, and human beings. Thus, it is necessary to develop environmentally friendly druthers to control soil pests, similar as entomopathogenic nematodes (EPNs). Since EPN species are host specific, they can be used widely for the target organisms. Today, the use of entomopathogenic nematodes as biopesticides against agricultural insect pests has grown quickly in recent times. These bio-pesticides play a great part in producing organic crops and export goods. So, researchers and advanced institutions should have to give attention in producing, formulating, and storing environmentally safe biopesticides. Nowadays, it is insolvable to ensure sustainable growth of crop yields without the application of fertilizers. Still, when using soil emendations, it is necessary to select the once that will round the living terrain to help negatively affecting the crops but also the structure and agrochemical conditions of the soil and the soil biota. Overall, it is apparent that nitrogen negatively affects the bacterial symbionts, but it is unknown whether or not potassium counteracts the toxin, or if the bacterial growth was affected by commodity differently. Results suggest that organic diseases brace better with *Xenorhabdus nematophila* and *Photorhabdus luminescens*, but they may warrant too important nitrogen for the shops to be suitable to produce.

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Conflict of interest

"The authors declare no conflict of interest."

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