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Entomopathogenic Nematodes: Integrated Pest Management and New Vistas

Mahfouz M.M. Abd-Elgawad[1], Weibin Ruan[2], Mostafa M.A. Hammam[1]

[1]Plant Pathology Dept., National Research Centre, Dokki, Giza, Egypt; [2]College Life Science, Nankai Univ., Tianjin 30071, China.

Corresponding author email:mahfouzian2000@yahoo.com

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ABSTRACT

Despite the current use of entomopathogenic nematodes (EPNs) commercially, there are still new prospects for expanding their applications to occupy a prominent market position. Scientists, extension specialists and stakeholders need to identify and widely disseminate conditions under which EPN application can offer a cost-effective, value-added approach to integrated pest management. Moreover, EPN use should not be limited to plant pests. There are other pests that EPNs can effectively and safely control such as those that significantly affect health and production of farm animals and honey bees. Examples of such pests against which nematodes can be reliably applied and general precautions to be taken to optimize EPN operation are given. The wide host range of EPN and their mutualistic bacteria against arthropods and pathogens are promising for advantageous industrial products for boosting pest/disease management. Therefore, a full useful spectrum of the EPN-bacterium complex or the symbiotic bacterium individually should be harnessed for useful usage in current and emerging agricultural systems. Fitting symbiont-obtained insecticidal, acaricidal, nematicidal, pharmaceutical, fungicidal, antimicrobial, and toxic compounds into current or emerging strategies, for controlling many pests/pathogens should be earnestly sought.

Keywords: Biological control, Entomopathogenic nematode, Pest and pathogen management, Production practices.

INTRODUCTION

Entomopathogenic nematodes (EPNs) were discovered in the 1920s. Since that time, the complex relationship between these nematodes in the two main genera *Steinernema* and *Heterorhabditis* and their bacterial symbionts (Poinar et al., 1977) formed a foundation of pioneering related research that has resulted in major advances in biological control as well as strides in basic studies. The two genera relate to two evolutionarily convergent families: Steinernematidae and Heterorhabditidae that are unified by their common use of symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively; the bacteria are critical for the exploitation of insect hosts. Notably, the two families have had evolutionary tactics to parasitism with their nutritional relevance to the bacteria. The practical operation and implications of this symbiosis are extraordinary. Contrary to other entomophilic nematode genera that are difficult to mass-culture, the mutualistic bacteria of EPNs convert numerous proteins into a diet/substrate in which the nematodes can readily develop and multiply. The resulting capability to easily generate huge numbers of EPNs via various *in vitro* production methods is responsible for their rapid progress in the past few decades from mostly-unknown insect parasites to broadly researched and commercially-available biological control agents (BCAs). However, other genera, *Oscheius* and

Neosteinernema, are also known as EPNs but do not practically serve as BCAs. Therefore, this review will only address species of *Steinernema* and *Heterorhabditis.*

Because of their high profile of safety to non-target organisms, humans, and the environment (Piedra Buena et al., 2015; Askary and Abd-Elgawad, 2017), EPNs have been largely exempted from pesticide registration matters in numerous countries. The lack of registration requirements contributed to EPN commercialization starting from the 1980s. Koppenhöfer et al. (2020) have recently recorded the commercial development of at least eight *Steinernema* species and five *Heterorhabditis* species, but cottage industry entities can also produce any additional species on demand. The inclusive body of EPN research has been presented and reviewed comprehensively from time to time in published books and articles (Gaugler and Kaya, 1990; Bedding et al., 1993; Gaugler, 2002; Grewal et al., 2005; Campos-Herrera, 2015; Abd-Elgawad et al., 2017; Shapiro-Ilan et al., 2020). Yet, this review will focus especially on additional topics that represent new horizons for expanding EPN utility to emerging pests of economic significance. Specifically, the use of EPNs for protection of pollinators and livestock is emphasized.

EPN Biology and Ecology

a) Foraging strategies of dauer juveniles within the foraging continuum

Due to the ongoing nematode surveys worldwide, the numbers of the EPN species and their mutualistic bacteria are always subject to increase. At least 102 *Steinernema* and 22 *Heterorhabditis* species have been identified to date (Bhat et al., 2020; Shapiro-Ilan et al., 2020; Hazir et al., 2022). EPNs have been isolated from all continents around the world except Antarctica. Also, their symbionts have recently been recorded as 20 *Photorhabdus* (Abd-Elgawad, 2021) and 27 *Xenorhabdus* species (Abd-Elgawad, 2022). A specialized third stage juvenile, usually called the dauer juvenile or infective juvenile (IJ) is the only free living stage that can actively invade the insect host. Foraging strategies of IJs have two different extremes within the foraging continuum; ambushers versus cruisers. For example, *S. carpocapsae* and *S. scapterisci* are ambushers (sit-and-wait) strategists that tend to wait near the soil surface using specialized host seeking behavior (nictation, jumping) to facilitate attacking mobile hosts. *Steinernema glaseri* and *H. bacteriophora* are at the other extreme where they widely search as cruisers for their hosts throughout the soil profile. Therefore, cruisers are generally well adapted to infect less mobile hosts. Notably, the majority of EPN species seem to be located somewhere within the foraging continuum between the two extremes.

b) Environmental factors

Admittedly, such foraging behaviors are adjusted by various factors such as soil texture and properties, volatile cues and signals from either their host insects or plant roots damaged by these herbivorous hosts (Shapiro-Ilan et al., 2018). On the contrary, cues released by IJs seem to adversely affect the performance of root insect herbivores (Helms et al., 2019), which may raise the benefits of using EPNs in IPM strategies. Ultimately, the non-feeding IJs utilize environmental and host cues in order to find and enter insects hosts. Infection into a host may be via natural openings (spiracles, mouth, and anus) or directly via penetrating thin parts found in the insect cuticle. Once established in the hemocoel, the highly specific link between EPN and bacterium begins to operate within the insect on releasing these bacteria via EPN regurgitation or defecation. The symbionts are routinely known to cause insect mortality via septicemia and or toxemia, within the context of the natural *Photorhabdus-Heterorhabditis* or *Xenorhabdus-Steinernema* complex. Nematodes and bacteria together can kill a susceptible insect within few hours to several days, mostly 1-3 days. While *Steinernema* spp. IJs grow into adults (males and females), and recycle within the host several times to generate males and females (with the exception of *Steinernema hermaphroditum*), *Heterorhabditis* spp. IJs develop to hermaphroditic adults in the first generation. However, their following generations comprise hermaphroditic individuals in addition to males and females. As the EPNs feed on both the bacterial cells and insect cadaver tissues digested by the bacteria, they usually can complete 1-3 generations before nutrient resources of the host cadaver are depleted within 1-4 weeks (Koppenhöfer et al., 2020). Usually, thousands of new IJs with the symbionts in their digestive system leave the infected cadaver to seek out new insect hosts. While leaving, *Steinernema* spp. IJs carry the symbiotic bacteria in a specialized vesicle in their anterior gut, whereas *Heterorhabditis* spp. harbor them linked to their pre-intestinal valve.

c) The role of mutulastic bacteria and their entomopathogenic nematode partners

Since their isolation by Poinar et al. (1977), the mutualistic bacteria have been intensively researched and proved to play a significant role in inducing mortality of the EPN-insect hosts via suppression of the insect-immune system. Poinar et al. (1977) suggested inclusion of the bacterial species isolated from *Heterorhabditis bacteriophora* in the genus *Xenorhabdus*, as *X. luminescens* (Thomas and Poinar, 1979). Later, the name *X. luminescens* was changed to *Photorhabdus luminescens* (Boemare et al., 1993). The mutualistic bacteria depend on their nematode partners to vector them from host to host. They can generate antibiotics that block secondary host invasions while offering a proper diet for the nematode feeding within the insect cadaver. The definitive role of the symbionts and nematodes in overcoming the insect host-immune response has only recently been investigated in some nematode-bacteria complexes for specific species of insect hosts. These studies revealed that the nematodes are also important partners in killing the insect host (Lu et al., 2017; Chang et al., 2019).

d) Challenges that face the application of entomopathogenic nematodes in integrated pest management

Early EPN-related studies are based on the assumption that these mutualistic bacteria have no infective capabilities and cannot persist outside the nematodes or insect host. However, based on recent references, Abd-Elgawad (2022) projected that a *Steinernema-Xenorhabdus* combination or *Xenorhabdus* individually should be included in mechanisms underlying the advantageous side of plant–phytonematode interactions in current and emerging cropping systems. Therefore, even *Xenorhabdus* bacterial species individually should earnestly be utilized to manage plant pathogens/pests within integrated pest management (IPM) programs. The importance of fitting *Xenorhabdus*-obtained insecticidal, antimicrobial, fungicidal, nematicidal, pharmaceutical, acaricidal, and toxic compounds into present and arising IPM strategies for management of numerous pests/pathogens was recently highlighted (Abd-Elgawad, 2022). Nonetheless, the relevant practical usage of *Xenorhabdus* spp. bacteria has not been swiftly progressing because of costs and issues related to their commercial processing. Likewise, but with more optimism due to a breakthrough in cheap production costs of *Photorhabdus* spp. singly (Keskes et al., 2021), *Photorhabdus-Heterorhabditis* combinations or *Photorhabdus* individually can be used for such different purposes and against many plant pathogens and pests (Abd-Elgawad, 2017a, 2021). The spread in using *Photorhabdus* spp. bacteria has also been slow-going, due to natural existence within the aggregated distribution of their EPN partners, and issues related to trait stability of their *in vitro* culture.

e) Mode of action and methods of application to control various pests/pathogens.

Despite such obstacles, significant advances have been achieved in terms of providing affordable *Photorhabdus*-mass production and mastered genome sequencing of both *Photorhabdus* spp. (Machado et al., 2021) and *Xenorhabdus* spp. (Castaneda-Alvarez et al., 2021), while detecting more of the beneficial species/strains of both genera (Abd-Elgawad, 2021, 2022). The high pathogenicity of *Photorhabdus* and *Xenorhabdus* species to a broad range of arthropods, effectiveness against certain pests and diseases, and versatility bode well for opening new horizons for their advantageous products (Bock et al., 2013; Wu et al., 2022). The numerous useful properties of such mutualistic bacteria can ease their integration to other management tactics/strategies for crop protection. To summarize, fields of usage, mechanism of action, and mode of application to control various pests/pathogens are shown for species of *Photorhabdus* (Figure 1) and *Xenorhabdus* (Figure 2).

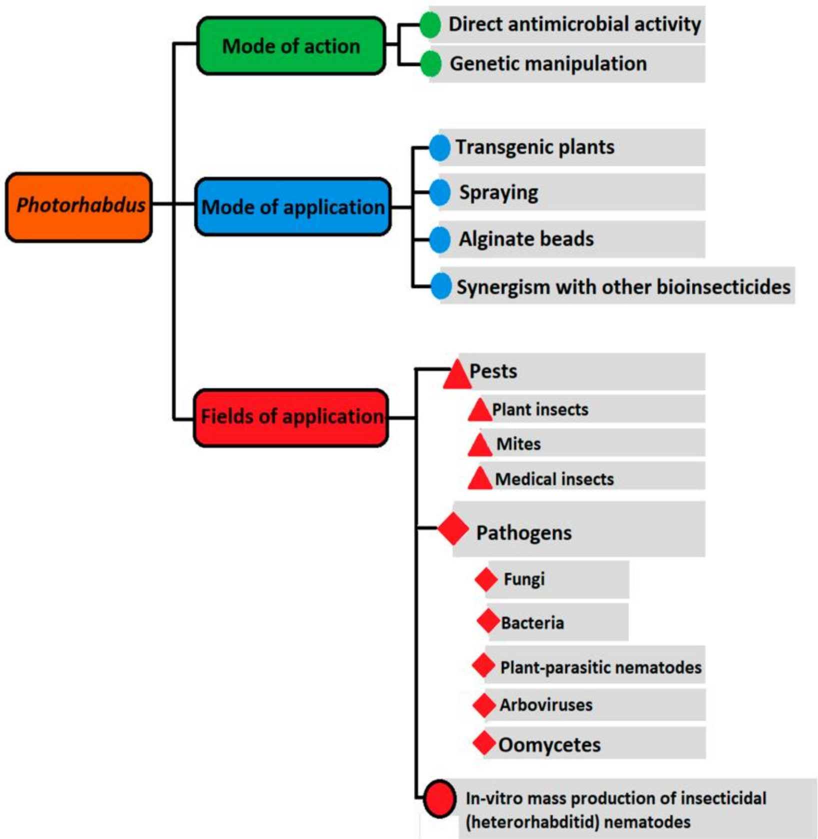

Figure 1. A sketch for possible methods and targets of application as well as mode of action for *Photorhabdus* bacteria in controlling pests and pathogens (Abd-Elgawad, 2021).

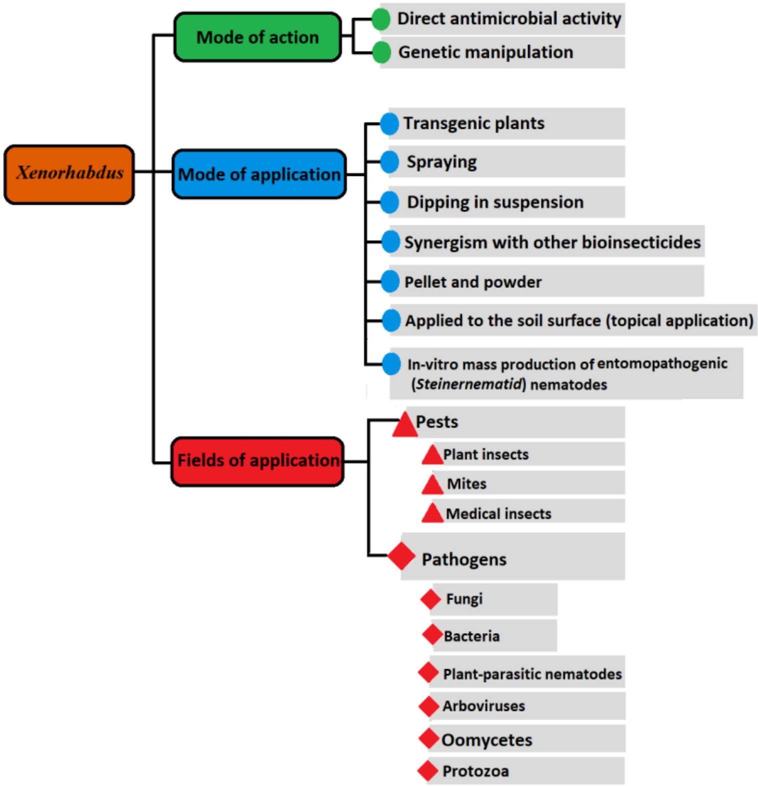

Figure 2. A sketch for possible methods and targets of application as well as mode of action for *Xenorhabdus* bacteria in controlling pests and pathogens (Abd-Elgawad, 2022).

General EPN Applications and Precautions

a) Nematode survival and persistence

Precise entomopathogenic nematode-host matching (i.e., choosing the appropriate nematode species) is necessary to achieve optimal field performance in the intended pest management arena. Additionally, ecological conditions must also be considered as well to achieve biocontrol success. For superior matching, it may be possible to determine from EPN characteristics the species/strain that is most likely to control certain insect species.

Nematode survival and persistence are key factors in biocontrol efficacy. Nematode persistence is based on the longevity of individual IJs and the reproductive capacity of EPN populations in insect cadavers, but many factors can modulate both attributes (Shapiro-Ilan et al., 2018; Koppenhöfer et al., 2020). Soon after EPN application, decline in nematode population can reach up to 50% until IJs have set in the soil. Thereafter, losses begin to slow to around 7% daily. Only about 1% of the original applied EPN populations survive at 1-6 weeks post-inoculation. Therefore, the standard application rate is 2.5 x 10^9 IJs/ ha or 1.05 x 10^9 IJs/ Feddan is required in order to compensate for such IJ mortality percentages. Moreover, certain pests or cropping systems may demand higher rates. Usually, applied IJ populations can stay in soil at population levels high enough to offer effective pest suppression for 2-8 weeks. Although EPN recycling after their introduction frequently occurs, it is not at a range sufficient to obtain multi-season control since IJ distribution are too aggregated over time. However, exceptions occur as well where multi-season control has been achieved (Shapiro-Ilan et al., 2020).

b) Precautions to avoid losses in nematode activity

In order to avoid extraordinary losses in nematode activity, several precautions must be considered. The EPN introduction should be done in the evening or early in the morning to avoid damage by UV radiation. Also, adding UV protectants to the IJ suspension and/or making soil applications in elevated carrier volumes linked to instant rinsing in with enough amounts of water can alleviate EPN losses. Optimal activity of most EPN species/strains are in the range 20-30 ˚C. Nonetheless, some EPN species/strains have been recovered from cold regions whereas others from hot semi-arid or even arid regions. Such uncommon species may hold promise for applications in corresponding environments.

Also, biotic factors should be considered. Many species of arthropods/ invertebrates can prey on the nematodes or feed on their infected hosts while other soil-borne pathogens can compete or synergize with EPNs for mutual hosts. Effects of edaphic and biotic factors on the nematodes were reviewed by Koppenhöfer et al. (2020). To optimize EPN benefits, certain combinations of control agents can be included in IPM programs, and trends that make them provide a cost-effective, value-added approaches were reviewed (Abd-Elgawad, 2017b, 2019; Stevens and Lewis, 2017). This is especially significant where EPNs can operate synergistically or additively with other agricultural inputs in IPM programs. Consolidated application of bio-pesticides and other chemicals should be practiced on a broader basis (Abd-Elgawad, 2019). This is an especially attractive approach, because there are numerous BCAs/bioactive compounds which are or are likely to be broadly available soon. Identification of research priorities for harnessing insecticidal nematodes in sustainable agriculture should always be updated.

New Vistas for EPN Applications

As aforementioned, clearly there are extensive studies on EPNs as BCAs embodied in many research papers and chapters of books. Such studies address insect species or groups and commodities/crops in which these nematodes have been experimented more intensely, each with several field trials showing good control potential by EPNs (Campos-Herrera, 2015; Abd-Elgawad et al., 2017; Koppenhöfer et al. 2020; Askary and Abd-Elgawad, 2021). On the other hand, scientific research always aspires to new research that challenges the recently recorded losses created by specific pests which were not of importance before at least in specific countries. This review on EPNs as BCAs will be limited to some of these less studied pests or only briefly described before as well as those that still lack safe and effective control methods to achieve sustainable development in agriculture sector. Heretofore, we focus especially on two less-studied opportunities for biocontrol using EPNs, pests of livestock and pests of pollinators.

Entomopathogenic Nematodes against Pests of Farm Animals

a) Problems facing the application of pest control

Although EPNs can offer excellent arthropod pest control in various situations, application is often not put into practice without adequate exploitation by stakeholders. For plant pest and pathogen control, such problems are mostly related to cost and reliability. For example, Dolinski et al. (2012) found that the potentially widespread utilization of EPNs in cotton and turf were replaced by insect resistant germplasm and less expensive chemical pesticides, respectively. Nevertheless, inferior effectiveness of EPNs relative to chemical insecticides in numerous situations and inconsistent operation of EPNs, based on product quality control or ecological variation, are the greatest hindrances to the adoption of EPNs in control schemes of many plant pests (Abd-Elgawad, 2017b; Abd-Elgawad and Askary, 2020). On the other hand, there are also instances where cost-effective pest control is achieved with EPNs at efficacy comparable or superior to chemical insecticides (Wong et al., 2022), Admittedly, pest management strategies and tactics continually evolve in response to issues comprising safety, pesticide resistance, development of pest biotypes that break host plant resistance, and health and environmental concerns. Such problems as well as technical advances in EPN mass-production and application can offer further chances to integrate EPN applications in pest management plans of many economically important resources. This is especially important for controlling pests/pathogens in agriculture sector such as those related to livestock and crops. Therefore, some researchers (Dolinski et al., 2012) could describe a few agricultural industries in Brazil, the United States, and Korea in which EPN applications have been examined and consequently recommended for arthropod pest control. In each case, they could conclude the basis on which EPN were adopted as well as the observed reasons for the present status of commercial EPN products. Also, Askary and Abd-Elgawad (2021) discussed the current opportunities and challenges of EPNs as biocontrol agents of many pests.

b) Pests and diseases of farm animals compared to plant pests in term of EPN application

Only recently, pests and diseases of farm animals are starting to receive attention comparable to plant pests in terms of EPN applications. This does not negate the related potential widespread use of EPNs or the fact that many arthropod pests can attack and inflict diseases on various species of livestock worldwide. Factually, current records reflect great economic losses in both milk and meat production of livestock due to pests/diseases. Such losses may be inflicted directly via the infection consequences in terms of less milk production and weight gain, anemia, and even animal death. Indirect losses are also big via transmitting disease-causing pathogens (Ghosh et al., 2007). For example, *Rhipicephalus microplus* Canestrini (the southern cattle fever tick) is broadly diffused in tropical and subtropical regions, causing big economic losses to meat and milk production. Chemical acaricides are widely applied as the main control strategy, therefore, there is augmenting evidence of resistance to acaricides in addition to mounting environmental concerns (Singh et al., 2018). Hence, novel strategies are desperately required. Use of EPNs proved to be a favorable alternative for tick control. In this respect, laboratory studies demonstrated that EPNs are a favorable alternative method to control *R. microplus* (Vasconcelos et al 2004, Monteiro et al. 2010a,b, 2014, Silva et al. 2012). Moreover, some EPNs proved to control other important pests of livestock, e.g. *Amblyomma americanum* (L.) (Kocan et al. 1998), *R.* (*B.*) *annulatus* Say (Alekseev et al. 2006, EL Roby et al. 2018), *Ixodes ricinus* (L.) (Grewal et al. 2001), *Anocentor nitens* Mohler (Monteiro et al., 2014), and *Argas persicus* (Oken) (Shamseldean et al. 2007, 2008; EL Roby et al. 2018). Utilizing EPNs against the non-parasitic phase of engorged female ticks can be quite efficient, since engorged females seek as moist environments protected from solar radiation as those settings favorable to EPNs (Grewal et al., 2001). Thus, these ecological conditions suit both EPNs and the females at the oviposition time. Livestock pests especially those including blood-sucking insects may greatly affect animal production (Kamut and Jezierski, 2014). They can also transmit numerous kinds of viruses, bacteria, and protozoa between domestic animals (Walker, 2017). Kamut and Jezierski (2014) mentioned various insects from six families of Diptera that harm farm animals worldwide. Based on promising but primary results (El-Sadawy et al., 2008a, b and 2018, Singh et al., 2018), applying EPNs as safe and effective bio-pesticides against these pests under field conditions should be a priority research area. Clearly, EPN viability (percentage of emerged living infective juveniles), infectivity (power to invade), reproductive capacity (yield per insect), and virulence (power to kill) may vary from one EPN species/strain to another (e.g., Shehata et al., 2019). Thus, infection of ticks by EPNs, as striking models of livestock pests, can vary depending also on the tick species, stage of tick development, EPN species or strain, and exposure time of the ticks (Samish et al., 2008). For instance, several records indicated effectiveness of *Steinernema* (Frietas-Ribeiro et al. 2005, Reis-Menini et al. 2008, Molina-Ochoa et al. 2009, Carvalho et al. 2010) and *Heterorhabditis* species (Monteiro et al. 2010a,b, 2012; Silva et al. 2012) against *R. microplus* with varying levels of virulence. In a more recent elaboration, six species of EPNs, i.e. *S. carpocapsae* (Weiser) (All strain), *S. riobrave* Cabanillas, Poinar, and Raulston (355 strain), *H. bacteriophora* Poinar (VS strain), *S. feltiae* Filipjev (SN strain), *H. floridensis* Nguyen, Gozel, Koppenhöfer, and Adams (K22 strain), and *H. indica* Poinar, Karunakar, and David (HOM1 strain) were tested for pathogenicity against the southern cattle fever tick (Deutch strain). Engorged female ticks were subjected to 1,250, 2,500, and 5,000 IJs for 24, 48, and 72 hours in Petri dishes, and tick mortality over time, pre-oviposition period, egg mass weight, reproductive index, percentage inhibition of oviposition, and hatching percentage were evaluated. Dose-dependent mortality of the ticks was reported in *Heterorhabditis* spp., *S. carpocapsae*, and *S. riobrave*. It was raised with exposure time. Minimum LC_{50} (95% confidence profile) values of 69 (63-75) and 1999 (1947-2052) IJs per Petri dish were recorded for *H. floridensis* and *S. riobrave*, respectively, at 72 hours of exposure. Nonetheless, neither exposure time nor concentration of IJs could affect the pre-oviposition period. Hatchability was lowered only in ticks exposed to *S. riobrave*. Overall, this study (Singh et al., 2018) showed *H. floridensis* as the most promising species in killing engorged *R. microplus* females and negatively influencing the majority of biological indexes. Others such as *S. riobrave* demonstrated potential since it is indigenous to South Texas and is marketed commercially. In field tests the EPNs provided control of the *R. microplus*; an automated "smart sprayer" was developed to treat the vertebrate animals, and moreover passive transfer from of the applications from the surrounding spray area also contributed to tick mortality (Goolsby et al., 2018; Goolsby and Shapiro-Ilan, 2020). Eventually, with increasing the host range of farm animal pests to include more susceptible host species (Esteve-Gassent et al., 2014) as well as the growing resistance of these pests to chemical and even biological pesticides (El-Ashram et al., 2019), the role assigned to EPNs has become more clear and urgent.

The Potential of EPN‑Based Biopesticides for Control of Honey Bee Pests

a) The importance of Honey bees for human wellbeing and the so called "forgotten economy"

Honey bees (*Apis mellifera*) are perhaps the most important insect for human well-being. The bees offer great favors via serving to pollinate more than $200 billion USD in agricultural crops yearly (Gallai et al., 2009). Moreover, sales of hive products can significantly make additional profits. Nevertheless, honey bees have been suffering considerable annual colony losses in numerous countries. American beekeepers alone lost over 50% of their colonies in 2018 (United States Department of Agriculture, 2019). Likewise, the number of hives has decreased in Egypt from about 1.40 million hives in 2000 to about 818 thousand hives in 2017; a decrease of about 42% during this period. Consequently, honey production decreased from about 8.21 thousand tons in 2000 to about 4.14 thousand tons in 2017 (El-Tatawy et al., 2019). Thus, there is a strong call not only to revive but to advance the so-called "forgotten economy." Honey bees and related products represent productive activities suitable for small and medium enterprises, and contribute to solving the problem of unemployment. This sector has several social, economic, health and environmental benefits. Although such high losses have been associated with several co-occurring biotic and abiotic factors (Harwood and Dolezal, 2020), pests of *A. mellifera* colonies can significantly contribute to these losses. They can adversely affect overall hive products, colony health, and productivity (Sanchez et al., 2021).

b) The greater wax moth *Galleria mellonella* and its impact on bee products and bee colony

The greater wax moth, *Galleria mellonella*, is familiar to any entomopathogenic nematologist because it is widely used as a model host and serves commonly as the primary choice for in vivo culturing of EPNs. This is due to its high susceptibility to infection by most EPN species/strains in addition to the ease, availability, and low-cost of insect rearing. This same insect is frequently reported to bring about damage both to bee products and to honey bee colonies in tropical and sub-tropical regions. Empty combs, comb foundation, rendered wax, and bee-collected pollen, if not adequately stored or left unattended, almost on all occasions may experience considerable damage from *G. mellonella* infestation, the insect may often cause bee colonies to escape (FAO, 2006).

The adult female of this *Galleria* often enters the hive at night via the entrance or cracks in the walls, deposits her eggs directly onto the combs or in narrow crevices that permit oviposition and offer prevention against elimination by worker bees (FAO, 2006). About 50-150 eggs are laid in each batch; usually glued together and seized tightly to the surface on which they are laid. The newly-hatched *G. mellonella* larvae feed on honey and pollen, and then burrow into pollen-storage cells or the outer edge of cell walls, later expanding their tunnels to the midrib of the comb as they develop. At this stage, the growing *G. mellonella* larvae are totally safe from the worker bees. Negatively, they leave behind them lumps of webs and debris while advancing into the combs. As a result, the complete destruction of unattended combs usually occurs within 10-15 days (FAO, 2006). Additionally, *Galleria* larvae can attack bee brood at food shortage. Food and temperature are key factors in determining the span of the pest life cycle.

Because there are no easy, safe, and cheap chemical insecticide for controlling *G. mellonella* in living honey bee colonies once infestation has initiated, the only possibility is via biocontrol agents. Treatment with *Bacillus thuringiensis*, in a watery suspension, sprayed onto the combs is recommended (FAO, 2006). Their effect on the wax-moth's larvae persists for several weeks. Using EPNs in a similar treatment may be tried too. Preventive measures comprise ensuring that the bee colonies are strong and have proper food stores; adjusting the hive space to the capacity of the colony; closing crevices and cracks in hive walls; minimizing the hive entrance; preserving the colonies from pesticide poisoning; controlling diseases/pests that might otherwise degrade the colonies; eliminating any wax/debris found on the bottom boards of the hives; and protecting hive products in tight, moth-proof rooms.

c) Small hive beetle (SHB) *Aethina tumida*

Another pest that is a good target to control via EPNs is the small hive beetle (SHB) *Aethina tumida* (Coleoptera: Nitidulidae). The pest is linked to beekeeping. It accomplishes most of its life cycle inside bee colonies but pupates in the soil under/around colonies (Zawislak, 2014). Adults of *A. tumida* usually fly to a suitable hive, mate, and lay eggs in clusters within the wax frames to start infesting *A. mellifera* colonies (Graham et al., 2011; Neumann and Elzen, 2004). After hatching, the larvae feed on pollen, honey, and *A. mellifera* brood while developing. Thus, the insects damage the frames, ferment honey, and create proper settings for other pests to increase (Neumann et al., 2013). Like the wax moth, food and temperature are key factors in determining the span of beetle life cycle (Neumann et al., 2013). The developed larvae always drop beneath the hive and start to seek out a proper pupation location, they are known as wandering larvae. Once in the soil, *A. tumida* develop into pupae and emerge as adults within 13-74 days (Neumann et al., 2013). EPN species efficacy in controlling SHB varies; Sanchez et al. (2021) tested six species against in three soil types, i.e. Kalmia loamy sand (KLS), Benndale fine sandy loam (BFSL), and Decatur silt loam (DSL). Of the six EPN species, *S. carpocapsae* recorded the highest efficacy across all EPN population densities tested in both autoclaved and natural soil. The nematode parasitized 87% SHB wandering larvae across all tested population levels and colonized 94% of the SHB in the KLS soil, 80% in the BFSL soil, and 47% in the DSL soil. Both *H. indica* and *S. riobrave* fulfilled the next highest efficacies. Sanchez et al. (2021) recommended *S. carpocapsae* as a promising BCA to be included in managing SHB.

Eventually, as the need for functional pollination by bees in numerous small fruit and orchard crops increases, utilizing EPN to control SHB seems of additional benefits. This is especially important as *A. tumida* is quite susceptible to EPN in both lab and field (Ellis et al., 2010; Shapiro-Ilan et al., 2010; Sanchez et al., 2021). Applying EPN-infested insect cadavers (*Tenebrio molitor*, infected with *H. indica*) recorded up to 78% control of *A. tumida* in the laboratory. Stevens and Lewis (2017) concluded that EPNs may be a straightforward control approach to use a small number of infected *T. molitor* cadavers (Dolinski et al., 2015) beneath hives to suppress mature *A. tumida* larvae moving into the soil.

Novel Advances to Boost EPN Efficacy

New developments are still needed to optimize EPNs efficacy given the above-mentioned examples to expand EPN use against emerging pests. Therefore, advances in strain development, mass production, formulation and application technology, combination with other agricultural inputs, and ecological manipulation can widen EPN usage (Shapiro-Ilan et al., 2017; Shehata et al., 2021). These factors must be worked on, starting with the methods of sampling and extraction to rationally broaden the genetic pool of the nematodes. In this respect, Abd-Elgawad (2020) proposed a sampling method for EPN with higher recovery and frequency values compared to other approaches, as well as precise distribution patterns based on combining four factors. These components comprised favorable sampling technique, site and time targeting and applying multiple extraction methods. This approach could both detect more EPN isolates and allow the use of various indices of dispersion to study the nematode-spatial distribution pattern. Following this approach, the nematodes were extracted from the seven surveyed groves (100%) and from 37 of 60 (61.7%) soil. Other approaches to boost efficacy will also be important such as improved mass production (Shapiro-Ilan et al. 2023), genetic improvement and stabilization of EPN strains (Shapiro et al., 1997; Shapiro-Ilan et al., 2017), formulation and application technology (Shapiro-Ilan et al., 2017, 2020) and efficacy boosters such as nematode pheromones (Oliveira-Hofman et al., 2019). Ultimately, we need to identify and broadly disseminate conditions under which the application of EPNs represents a cost-effective, value-added approach to IPM programs. We need to find out the slot where EPNs are the optimal tool in the IPM arsenal. Furthermore, we should seek scenarios in which EPNs can effectively and safely achieve things that other approaches cannot.

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الملخص العربى

النيماتودا الممرضة للحشرات: الإدارة المتكاملة للآفات والآفاق الجديدة

محفوظ محمد مصطفى عبد الجواد[1] - ويبين روان[2] – مصطفي محمد عطية همام[1]

[1]قسم أمراض النبات - المركز القومى للبحوث - الدقى 12622 - الجيزة - مصر.
[2]كلية علوم الحياة - جامعة نانكاي - تيانجين 30071 - الصين.

على الرغم من الاستخدام الحالي للديدان الخيطية (النيماتودا) الممرضة للحشرات تجاريًا ، لا تزال هناك آفاق جديدة لتوسيع تطبيقاتها لتحتل مكانة بارزة في السوق. يحتاج العلماء والمتخصصون في الإرشاد وأصحاب المصلحة إلى تحديد ونشر الظروف التي يمكن أن يوفر بموجبها تطبيق هذه النيماتودا على نطاق واسع نهجًا فعالاً من حيث التكلفة والقيمة المضافة للإدارة المتكاملة للآفات. علاوة على ذلك، لا ينبغي أن يقتصر استخدام هذه النيماتودا على آفات النباتات. هناك آفات أخرى يمكن للنيماتودا السيطرة عليها بشكل فعال وآمن مثل تلك التي تؤثر بشكل كبير على صحة وإنتاج حيوانات المزرعة ونحل العسل. تم إعطاء أمثلة على هذه الآفات التي يمكن استخدام هذه النيماتودا ضدها بشكل موثوق والاحتياطات العامة التي يجب اتخاذها لتحسين فعاليتها. يعد مجموع العوائل الواسعة لهذه النيماتودا والبكتيريا المتكافلة معها خاصة استخدامها ضد مفصليات الأرجل ومسببات الأمراض واعداً بمنتجات صناعية مفيدة لتعزيز إدارة الآفات والأمراض. لذلك، يجب تسخير السلسلة المتصلة لكامل تأثيراتها المفيدة سواء أكانت هذه النيماتودا متحدة مع البكتيريا المتكافلة معها أو البكتيريا التكافلية بشكل فردي في النظم الزراعية الحالية والناشئة. يجب البحث بجدية عن منتجات المبيدات التي يتم الحصول عليها من هذه البكتيريا المتكافلة معها مثل المبيدات الحشرية، ومبيدات القراد، ومبيدات نيماتودا النبات، والمركبات الصيدلانية، ومبيدات الفطريات، ومضادات الميكروبات، والمركبات الأخري سواء في الاستراتيجيات الحالية أو الناشئة، لمكافحة العديد من الآفات ومسببات الأمراض.