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From soil to host: Discovering the tripartite interactions between entomopathogenic nematodes, symbiotic bacteria and insect pests and related challenges

Najwa Seddiqi Kallali [a,b], Abderrahman Ouijja [b], Khadija Goura [a,b], Salah-Eddine Laasli [a], Jihane Kenfaoui [d], Youssef Benseddik [e], Abdelali Blenzar [b], Abdelmalek Boutaleb Joutei [e], Moussa El Jarroudi [c], Fouad Mokrini [f], Rachid Lahlali [a,*]

[a] Phytopathology Unit, Department of Plant Protection, Ecole Nationale d'Agriculture de Meknès, Km10, RteHaj Kaddour, B.P. 11201 Zitoune, Meknes 50001, Morocco
[b] Laboratory of Biotechnology and Valorization of Phyto-Resources, Faculty of Sciences, Moulay Ismail University of Meknes, Avenue Zitoun, Meknes 50001, Morocco
[c] Water, Environment and Development Unit, Department of Environmental Sciences and Management, UR SPHERES Research Unit, University of Liège, 6700 Arlon, Belgium
[d] Induced Resistance and Plant Bio-Protection Research Unit-EA 4707-USC INRAE1488, Reims Champagne-Ardenne University, Reims, France
[e] Entomology Unit, Department of Plant Protection, Ecole Nationale d'Agriculture de Meknès, Km10, RteHaj Kaddour, BP S/40, Meknes 50001, Morocco
[f] Biotechnology research unit, Regional Center of Agricultural Research of Rabat, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco

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ABSTRACT

Entomopathogenic nematodes (EPNs) are emerging as key agents in ecological networks, exhibiting a wide range of interactions with other biotic components, in particular their symbiotic relationships with the bacteria *Xenorhabdus* and *Photorhabdus*. This comprehensive study reveals their global distribution and local benefits and highlights their historical background and taxonomic grouping. The importance of the secreted compounds of EPNs in pest management is highlighted by an in-depth exploration of their potential as biocontrol agents. The complex interactions between nematodes and endosymbiotic bacteria are dissected to understand their mutualistic relationships and subsequent effects on host organisms. The strategies used by EPNs to locate, recognize, and invade hosts will be carefully analyzed to understand their pathogenic phase and the resulting immune responses elicited in insect hosts. Infection strategies employed by the EPN-bacteria complex will be examined to assess their efficacy and real-world challenges. The challenges associated with the effective use of EPNs, including environmental constraints and the need for improved efficacy, will be thoroughly investigated to propose viable solutions. This study paves the way for harnessing the biocontrol potential of EPNs and provides a robust framework for future research to improve the efficacy of EPNs in sustainable agriculture and pest management while addressing the challenges identified.

1. Introduction

Entomopathogenic nematodes (EPNs), principally from the families Steinernematidae and Heterorhabditidae, have been recognized as powerful biological control agents (Nurashikin-Khairuddin et al., 2022). The global distribution of EPNs is a sign of their adaptability and resistance. Surveys around the world have identified a plethora of EPN species, each with its unique attributes and potential applications in pest management (Tarasco et al., 2023). However, their ecological importance goes beyond simple insect predation. EPNs have multiple roles in soil ecosystems, influencing nutrient cycling, soil health, and plant productivity (Bhat et al., 2020; Tarasco et al., 2015).

The EPNs are not the only tools being used in the control of insect pests. Instead, they form a synergistic combination with specific bacteria, mainly from the genera *Xenorhabdus* and *Photorhabdus*, creating a beneficial duo that has attracted considerable attention in the field of biological control (Çimen et al., 2014; Dubey et al., 2013). This partnership, which has been developed for millennia, highlights a remarkable interaction between biology and ecology and constitutes a sustainable alternative to chemical methods of pest control.

* Corresponding author.
E-mail address: rlahlali@enameknes.ac.ma (R. Lahlali).

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Once inside the insect host, the bacterium produces a plethora of toxins that rapidly neutralize the host, paving the way for the nematode to reproduce (Özdemir et al., 2020). The term 'entomopathogenic' for these nematodes therefore indicates this double mechanism of action in which both the nematode and its bacterial symbiont have an important role in insect disease (Lewis et al., 2006). Furthermore, the bacteria associated with EPNs, in particular *Xenorhabdus* and *Photorhabdus*, are not mere passengers in this symbiotic relationship (Stock, 2015). Their role in EPN pathogenicity is crucial and they have been the subject of extensive research, particularly concerning their toxin profiles and their potential applications beyond pest control (French-Constant et al., 2007; Hinchliffe, 2013). Recent studies have highlighted the entomopathogenic potential of these bacteria, even in the absence of their nematode partners, underlining their polyvalence and potential as independent biological control agents (Gümüşsoy et al., 2022).

However, the transition from soil to insect host involves a succession of events full of challenges and difficulties (Stock and Blair, 2008). EPNs and their bacterial partners have developed a series of strategies for localizing, recognizing, and infecting their hosts (Abdel Gawad et al., 2023; Nguyen and Hunt, 2010). The host insect, in its turn, is not a passive victim. Insects have developed a complex immune response to push back the bacterial invasions of EPNs, by creating several mechanical and chemical barriers against the attack and ensuring their defense (Loulou et al., 2023). Understanding the interactions between this EPN-bacteria-insect tripartite is crucial for optimizing the use of EPNs in biological control programs.

Several findings have underscored the effectiveness of EPNs in controlling various insect pests in different environments. For instance, Kotsinis et al. (2023) investigated the effect of four terpenes on the viability of EPNs, which is crucial for their integration into Integrated Pest Management IPM. Moreover, Spescha et al. (2023), showed that a consortium of biocontrol agents, including EPNs, increased the mortality of leaf and root-feeding pests, demonstrating the potential of collaborative biological control approaches. Also, in another study, a specific EPN strain, *Steinernema glaseri* (Steiner, 1929), was found as highly effective against a major walnut pest in China under laboratory conditions (NanGong et al., 2022). Recently, a study by Tomar et al. (2022) revealed the biocontrol potential of EPNs and their endosymbiotic microbes for agro-environmental sustainability, highlighting their efficacy against a wide range of harmful organisms and reducing the need for chemical pesticides. Nevertheless, the potential of EPNs is not without its challenges. Environmental factors, host specificity, and potential resistance mechanisms in target pests are just some of the obstacles that need to be overcome to exploit the full potential of EPNs and their symbiotic bacteria. However, EPNs and their symbiotic bacteria present the world with a rich array of interactions, challenges, and opportunities (Askary and Abd-Elgawad, 2021). As we delve deeper into their biology and ecology further, we are on the verge of revolutionizing pest control strategies.

In this review, we will examine the potential of EPNs and their symbiotic bacteria, which has captivated the interest of researchers, especially in the last ten years. However, a knowledge of the bio-ecology of nematodes, and an identification of the potential of these symbiotic bacteria and their properties (insecticidal, nematicidal, fungicidal, acaricidal, pharmaceutical, antimicrobial, and toxic) has proved important for a thorough understanding of these two minuscule organisms (Fig. 1). In addition, we will explain in detail the process of insecticidal potential exercised by the EPN-endosymbiotic bacteria complex. This complex arises from a synergetic symbiosis, which infects and exploits these hosts through a succession of steps starting with the contribution of strategies for searching and recognizing the host before penetrating it. Along the same theme, we have highlighted the tripartite interactions that take place between the nematode-bacteria duo and their insect hosts. Furthermore, we will discuss the importance of EPNs as a biocontrol strategy against pests that face several biotic and abiotic challenges and obstacles. Indeed, for sustainable exploitation of EPNs, we suggest the need for very thorough research on the different nematode species as well as their association with their host range. This will enable the challenges to be overcome and the use to be improved and optimized to promote the insecticidal potential of EPNs in the agricultural sector.

2. Entomopathogenic nematodes

2.1. Ecological Interactions and significance of entomopathogenic nematodes

In nature, the ongoing interactions among organisms sharing the same ecological niche have given rise to various associations, which can be either beneficial or harmful. Nematodes serve as a highly illustrative model for a wide array of associations with both macro and microorganisms. Nematodes evolve through continuous interactions with bacteria, which can be beneficial (mutualistic), harmful (parasitic and pathogenic), or exhibit stable/transitory relationships (symbiotic) (Lacey et al., 2015; Murfin et al., 2012).

Throughout their evolutionary history, over 30 families of nematodes have adopted different types of interactions with arthropods, including mutualistic (symbiosis, phoresis, commensalism) or antagonistic (parasitism and pathogenesis) relationships (Lacey et al., 2015; Lacey and Georgis, 2012). However, most research in this field has predominantly focused on 10 families due to their significant insecticidal potential. These families encompass Allantonematidae, Aphelenchoididae, Mermithidae, Neotylenchidae, Rhabditidae, Sphaerularidae, Tetratonematidae, Phaenopsitylenchidae, Heterorhabditidae, and Steinernematidae (Grewal et al., 2005; Nguyen and Hunt, 2010).

The *Nematoda* is renowned for numerous taxa that have proven their effectiveness in biological pest control. However, among the insect-parasitic nematodes, EPNs belonging to the Rhabditida order, specifically Heterorhabditidae and Steinernematidae families, stand out as the most extensively employed in biocontrol, particularly against destructive insect pests (Dillman, Chaston, et al., 2012; Nguyen and Hunt, 2010). The significance of these two prominent families can be attributed to several key characteristics of Entomopathogenic Nematodes. First, they are non-harmful to humans, non-target organisms, and the environment (Koppenhöfer et al., 2020). Moreover, they demonstrate a remarkable ability to adapt and thrive in subterranean conditions (Rumbos and Athanassiou, 2017). EPNs have effectively demonstrated their insecticidal potential across a broad spectrum of insects, encompassing more than five taxonomic orders (Mokrini et al., 2020; Vicente-Díez et al., 2021; Yang et al., 2012). They establish symbiotic associations with bacteria, that facilitate the secretion of dangerous toxins, typically leading to insect death within 48 h (Tarasco et al., 2023). In addition, these small organisms have a short life cycle, and both *in vitro* and *in vivo* production methods are cost-effective. The commercialization of EPNs has even minimized the use of chemical pesticides in several European countries and North America (Askary and Abd-Elgawad, 2021; Dillman, Chaston, et al., 2012; Lacey et al., 2015).

Entomopathogens is a term commonly used in phytopathology and parasitology, which refers to the ability of a microorganism to induce diseases and mortality in its insect hosts. Interestingly, the term 'entomopathogenic' had a limited presence in the field of nematology until around 1980. Its first application began in 1981, however, the term gained substantial recognition until 1986. The application of "entomopathogenic" to the Steinernematidae and Heterorhabditidae is justified by these nematodes swiftly dispatching their hosts within almost 48 h (Dillman, Chaston, et al., 2012; Ivezić, 2020). These two families are obligate parasites of insects, with third-stage individuals known as "infective larvae" being the only forms capable of initiating infection, surviving, and persisting in the external environment. They achieve this by retaining the cuticle from the second juvenile stage in the form of a sheath (Addis et al., 2016; Susurluk et al., 2007). The infective larvae of

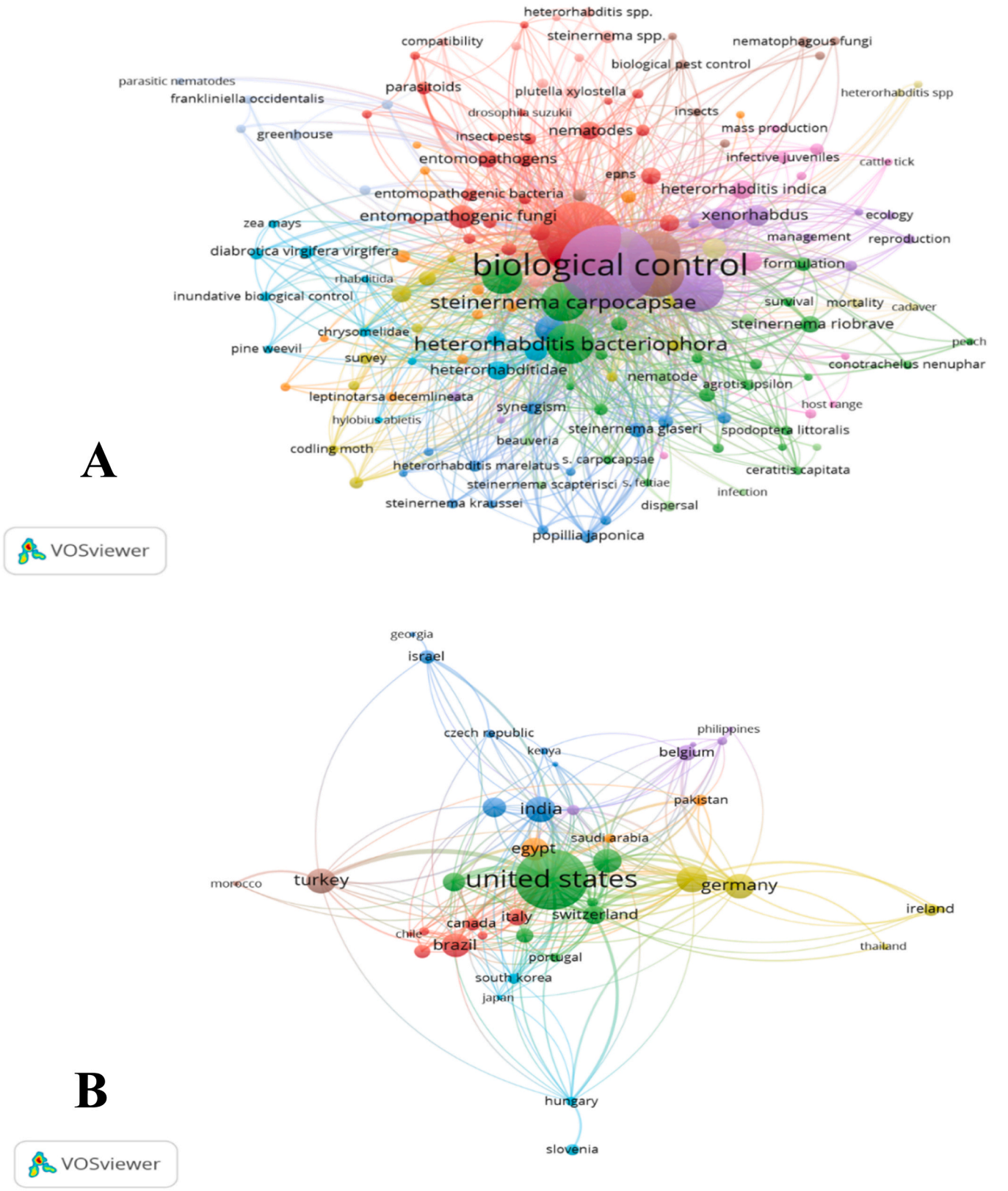

Fig. 1. Bibliometric analysis of 1308 articles published on the control of insects by the use of entomopathogenic nematodes and their bacteria according to the Scopus database using specific keywords such as “nematodes”, “entomopathogenic”, “pests”, and “biocontrol” (A) view online. The network analysis of EPN worldwide distribution (B) view online. The larger the circle, the more intense the scientific activity.

Heterorhabditis and *Steinernema* carry *Photorhabdus* and *Xenorhabdus* bacteria in their intestines (Fuenmayor et al., 2021; Karthik Raja et al., 2021; Pratissoli et al., 2021).

The relationship between EPNs and their symbiotic bacteria is one of obligatory mutualism, where each partner depends on the other for development, often at the expense of a suitable insect host. The nematode relies on bacteria to overcome the host insect's defense mechanisms and protect the insect cadaver from saprophytic microorganisms. In addition, the nematode uses the bacterium as a substrate for growth and reproduction. The bacterium is closely associated with the nematode, serving as a means of transport into the insect hemolymph and for survival in the external environment (Brivio and Mastore, 2018; Noujeim et al., 2016; Pervez et al., 2020).

2.2. Worldwide range and local utility of entomopathogenic nematodes

EPNs are cosmopolitan organisms, widely distributed worldwide. They are found on all continents except Antarctica, due to their intolerance of the psychrophilic conditions prevalent there (Lazarova et al., 2021). Currently, the genus *Steinernema* comprises more than 125 species, while the genus *Heterorhabditis* includes 22 species, that have been identified and distributed across different regions of the globe (Fig. 2) (Bhat et al., 2023; Sudhaus, 2023). These nematodes have successfully demonstrated their insecticidal potential, making them valuable tools for insect biocontrol. On a global scale, several studies have indicated that *Steinernema feltiae* (Filipjev, 1934) and *S. carpocapsae* (Weiser, 1955) are among the most widespread species, particularly in temperate regions. *Heterorhabditis bacteriophora* (Poinar, 1976), in particular, is ubiquitous in continental and Mediterranean climates. However, certain species have more limited distribution, such as *S. cubanum* (Mráček, Hernández & Boemare, 1994) and *S. riojaense* (Půža, 2020) (Bhat et al., 2020; Půža et al., 2020; Tarasco et al., 2015).

Geographically, EPNs are distributed throughout the American continent and can adapt to diverse ecological environments. The identification and description of numerous species of entomopathogenic nematodes in Latin America has been the result of extensive research. They are used to control more than 170 species of agricultural and urban pests in the region, proving the diverse potential of entomopathogenic nematodes for biological control (San-Blas et al., 2019). In North America, particularly in Canada, studies in areas like Ontario and Quebec have shown that EPN species such as *Steinernema carpocapsae, S. feltiae, and S. kraussei* are able to adapt to these climatic conditions (Simard et al., 2007). Furthermore, research by Bhat et al. (2020) highlights the presence of EPN species both native and introduced to North America, particularly Mexico, the United States, Cuba, and Costa Rica, as well as other countries. Species such as *Heterorhabditis bacteriophora*, *H. megidis, S. carpocapsae,* and *S. riobrave* have demonstrated efficacy in pest control. Some of these species were even commercialized (Bhat et al., 2020; San-Blas et al., 2019).

Extensive surveys in Europe have revealed a significant presence of EPNs, including species such as *S. feltiae, S. kraussei, H. megidis,* and *H. downesi.* These species are present in countries such as the UK, Germany, and France, where they are used in a variety of agricultural contexts, particularly in temperate climates. However, the different climate zones in Asia host different EPNs such as *S. carpocapsae, S. riobrave, S. abbasi,* and *H. indica* in India and mainly *H. beicherriana* in China. Owing to its diverse climates, the continent has been a focal point for discovering many EPN species (Bhat et al., 2020).

Moving to Africa, the continent's vast and diverse landscapes have led to the discovery of many new and known EPN species. Different climatic zones provide a range of habitats for these nematodes. Australia's unique ecological conditions have contributed to the discovery of species such as *S. feltiae, S. glaseri, S. kraussei, S. longicaudum, H. zealandica* and *H. bacteriophora,* which have adapted to the region's specific environmental conditions. While research in New Zealand is more limited, the presence of species like *S. kraussei, S. feltiae,* and *H. zealandica* suggests the possibility of specific EPN adaptations in the area (Bhat et al., 2020).

The continental distribution of EPNs demonstrates their ecological adaptability and their importance in integrated pest management strategies for a variety of pest species in different agricultural and urban settings (Fig. 2).

At the national level, the presence of EPNs in Moroccan agroecosystems has been documented in several studies. For instance, research by Benseddik et al. (2020), revealed the existence of EPNs in the soils of three regions in Morocco (Gharb, Saïs, and Middle Atlas)

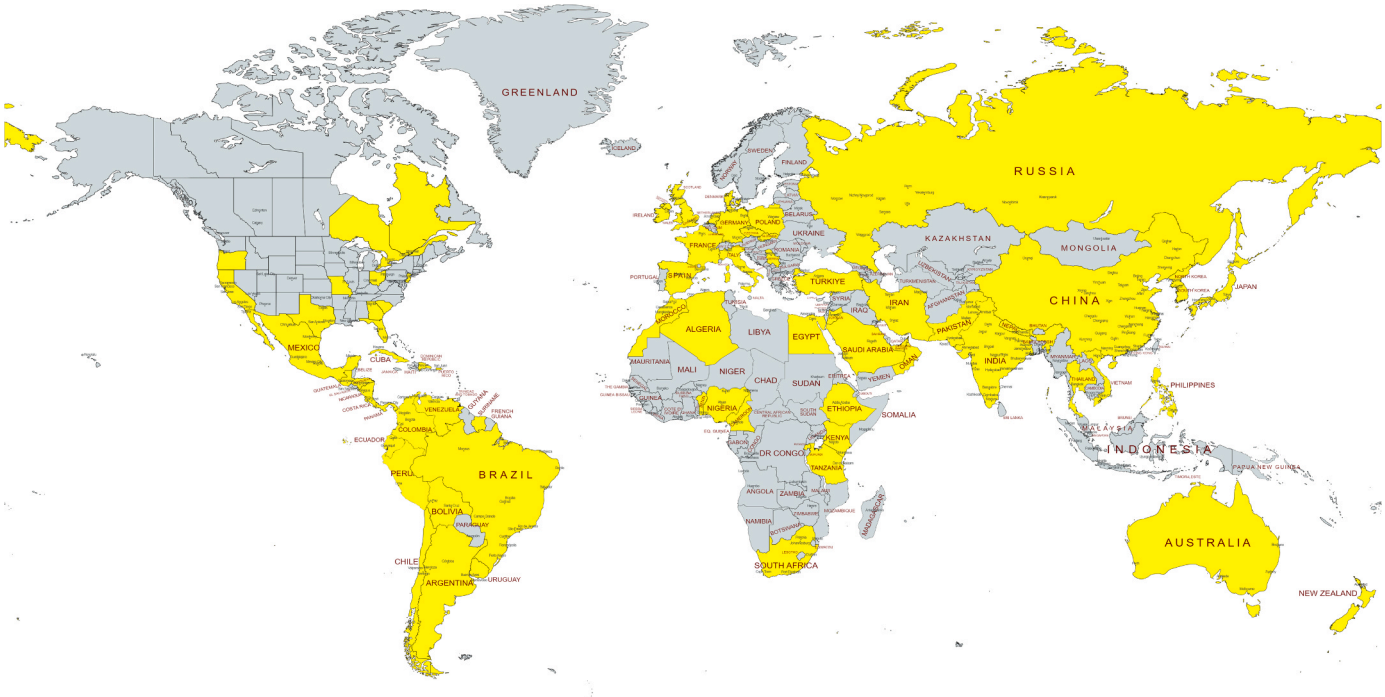

Fig. 2. Geographical map showing the general distribution of entomopathogenic nematodes reported on a global scale.

(Benseddik et al., 2020). Soils hosting these nematodes varied in texture and maintained a pH ranging from neutral to slightly alkaline. In a recent study, the effectiveness of 14 local strains of EPNs against the white grubs *Geotrogus olfersii* Fairmaire and *Rhizotrogus obesus* Lucas (Coleoptera: Scarabaeidae) was evaluated. These strains were isolated from the regions of Meknes-El Hajeb, Ifrane, and Tafilalet, showing remarkable success in controlling these white grubs (Benseddik et al., 2021). The significant presence of EPNs at the local level, combined with these outstanding results, suggests that these species could be an effective means of biological control.

In the same context, Mokrini et al. (2020), highlighted in a study the efficacy of five Moroccan ENP strains against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae). In laboratory tests, three strains, namely *S. feltiae* (SF-MOR9), *S. feltiae* (SF-MOR10), and *H. bacteriophora* (HB-MOR7), showed infectivity and penetrate rates than the other strains. Specifically, SF-MOR9 caused the highest larval mortality (80%) at a density of 50 infective larvae per square centimeter. Further tests showed that both *S. feltiae* strains were effective in controlling *C. capitata* larvae in apricot (*Prunus armeniaca*) fruit at the soil surface. Mortality rates were high at densities of 50 and 100 IJ per square centimeter. Additional trials showed that both *S. feltiae* strains were effective in controlling *C. capitata* larvae in apricot fruit at the soil surface, with high mortality rates at densities of 50 and 100 IJ per square centimeter. The virulence of EPN strains against *C. capitata* varied considerably with soil texture and moisture content. The sandy soil, together with 50 IJ per square centimeter density of *S. feltiae* (SF-MOR9 or SF-MOR10), resulted in higher *C. capitata* larval mortality. Furthermore, optimal results against *C. capitata* larvae were obtained when these EPN strains were applied at a density of 50 to 100 IJ per square centimeter in combination with a soil humidity of 10 to 15%. Therefore, these two Moroccan EPN strains are proposed as promising organic biological agents for the control of *C. capitata* (Mokrini et al., 2020).

In addition, a study realized by El Aimani et al. (2021) showed the efficacy of indigenous EPNs against the larval stage of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), was evaluated under laboratory and field conditions in Morocco. Laboratory tests showed that two strains of *S. feltiae* (SF-MOR9 and SF-MOR10) had significantly higher infectivity rates than the *Heterorhabditis* strains after 72 h. A bioassay showed that these strains, together with *H. bacteriophora* (HB-MOR8), caused the highest mortality (80–100%) at a given dose of infective juvenile DJs per square centimeter, highlighting the importance of the dose applied. The EPNs were able to localize the larvae precisely and caused mortality of the larvae in both internal and external conditions. In pot experiments and field trials, these EPN strains proved effective at certain applied concentrations, achieving mortality rates of over 80% under field conditions at specific doses and reducing pest presence by 60% at the same dose. This suggests the potential of these indigenous EPN strains as promising ecological biological control agents for the management of *T. absoluta* in tomato crops and highlights the importance of the dose applied to achieve effective pest control (El Aimani et al., 2021).

In the same context of using EPNs as a biological alternative to chemical pesticides, El Aalaoui et al. (2022) used two Moroccan EPN isolates, *S. feltiae,* and *H. bacteriophora*, were evaluated for efficacy against *Dactylopius opuntiae* (Cockerell, 1896) (Hemiptera: Dactylopiidae). *S. feltiae* was highly effective, producing mortality rates of 98.8% for nymphs and 97.5% for adult females within 8 days. On the other hand, *H. bacteriophora* produced lower mortality rates of 83.8% and 81.3% for nymphs and adult females respectively. There was no significant difference in the mortality rates when compared to the positive control, d-limonene, which is known to be effective against *D. opuntiae*. The potential of *S. feltiae*, which reduced pest populations from 85.3% to 93.9% in 12 days, was also confirmed in field trials. These results highlight the potential of Moroccan EPN isolates as alternative biological control agents against *D. opuntiae*, providing a sustainable solution for the control of this pest. The comparable efficacy of *S. feltiae* with d-limonene highlights its potential in integrated pest management strategies (El Aalaoui et al., 2022).

Collectively, the case studies highlight the potential of indigenous EPN strains as promising ecological biocontrol agents for managing various pests in Moroccan agriculture. The viability of EPNs as a sustainable pest management solution is underlined by the significant mortality rates observed for various pests and the emphasis on the importance of dose and soil conditions for effective pest control. Further research is needed to optimize EPN application methods and to integrate these biological pesticides into more comprehensive management strategies to improve agricultural productivity and sustainability in Morocco and similar agro-ecosystems.

2.3. Biology of entomopathogenic nematodes

Nematodes are multicellular worms of varying sizes, generally small to medium-sized, unsegmented, and typically slender in shape, exhibiting remarkable diversity in their forms and functions (Basyoni and Rizk, 2016). The life cycle of EPNs and their symbiotic bacteria comprises several stages, namely, there is an egg stage, four juvenile stages, and an adult stage. EPNs primarily feed and reproduce within the host cadaver throughout their life cycle. It is essential to note that only Dauer juveniles (DJ) also known as infective juveniles (IJs), which are the third life cycle stage of particular interest for biological control (Aumann and Ehlers, 2001). The DJ or IJ stage is a non-feeding, developmentally arrested stage adapted to environmental survival and host selection. This stage is characterized by its ability to withstand environmental stresses such as desiccation and a lack of nutrients (Addis et al., 2016; Campbell and Kaya, 2002). Indeed, *Heterorhabditis* and *Steinernema* nematodes utilize DJ as their mode of transmission from one host to another. These DJ stages are robust and can persist in the soil until they find a new host, entering through natural openings found in the insect's anatomy, including the mouth, anus, cuticle, and spiracles. Within these DJ stages, there exists a population of 200 to 2000 symbiotic bacterial cells within their intestines (Forst and Clarke 2002a, 2002b). Upon successfully entering the insect host, the DJ stages respond to specific chemical cues, known as "feeding signals." For instance, in the case of *Steinernema* spp., they release their symbiotic bacteria into the insect's hemocoel through the anus, while *Heterorhabditis* spp., release theirs through the mouth (Griffin et al., 2000; Ponnusamy and Belur, 2015). After this interaction, the DJ stages exit the dauer stage, a developmental phase that is stopped, due to the influence of the insect's hemolymph-derived feeding signals. Then, they resume their feeding activities, marking the onset of a critical developmental process known as DJ recovery. Interestingly, the terminology "DJ recovery" was initially introduced in the context of *Caenorhabditis elegans* (Golden and Riddle, 1984). Subsequently, these recovered DJ stages progress into the fourth juvenile stage (J4). The life cycle is finished in a few days, and countless numbers of new IJs develop, and start seeking new hosts. The cycle from IJ entrance into a host to the appearance of additional IJs is influenced by temperature and differs between taxa and strains. In most cases, the life cycle of EPNs (infective juvenile entry to infective juvenile release) is achieved within 12–15 days. The ideal temperature for growth and multiplication of nematodes is between 25 °C and 30 °C (Devi, 2018).

2.4. Comparative biology of steinernematidae and heterorhabditidae

The Steinernematidae family engages in symbiosis with bacteria belonging to the *Xenorhabdus* genus, which colonize their bilobed intestinal vesicle (Ciche et al., 2006; Sabbahi et al., 2022). Steinernematidae release their bacterial payload into the host via the anus, and during an infection of insects, they rupture the insect's cuticle, leading to the organism's rupture and, consequently, the discharge of a viscous liquid laden with these bacteria (Askary et al., 2018a; Boemare, 2002). In contrast, the Heterorhabditidae family, also living in symbiosis,

associates with bacteria of the *Photorhabdus* genus (Ciche et al., 2006; Malan et al., 2014). These bacteria predominantly inhabit the entire intestine, with a significantly greater presence in the anterior portion. During infection, the insect's cuticle remains intact (Ehlers, 2008; Stock and Goodrich-Blair, 2012). One of the distinguishing features facilitating differentiation between the two families is the external pore (SE) used for excreting nematode waste (Ehlers, 2008). The SE-pore's location sets the two families apart, as it is found posterior to the nerve ring in Heterorhabditidae, whereas it is located anteriorly in Steinernematidae. Additionally, the presence or absence of teeth on the heads of the infective juveniles in Heterorhabditidae is noteworthy, as these teeth are used to penetrate the host through its cuticle (Flores et al., 2021).

Furthermore, reproduction patterns differ between the *Steinernema* and *Heterorhabditis* species. In *Steinernema* spp., reproduction is amphimictic, meaning that "J4″ individuals can develop into both males and females excluding *S. hermaphroditum* (Rahoo et al., 2019) Conversely, in *Heterorhabditis* spp., reproduction follows a heterogonic pattern, where J4 individuals derived from DJ consistently transform into hermaphrodites. Nematodes, in their entirety, undergo complete development and reproduction, often spanning two or more generations within the host environment. Notably, when food resources become scarce and the nematode population multiplies, the DJs juveniles in this stage (referred to as J2ds) encounter colonization by their symbiotic bacteria. This process leads to their complete transformation into the infective DJ stages, ensuring the continuation of the nematode life cycle (Fig. 3) (Addis et al., 2016; Sikandar et al., 2021).

3. Entomopathogenic bacteria

Two families of nematodes in the order Rhabditida, Steinernematidae, and Heterorhabditidae, encompass organisms in their digestive systems, whose larvae can invade living insects and destroy them while growing in their cells (Han and Ehlers, 2011). *Brevibacillus*, *Bacillus*, *Pseudomonas*, *Peanibacillus*, *Serratia*, *Xenorhabdus*, and *Photorhabdus* represent the most common entomopathogenic bacteria found in nature (Ciche et al., 2006; Koppenhöfer and Gaugler, 2009; Ruiu et al., 2022). These entomopathogenic bacteria are known to have multiple host spectrum and infection pathways, depending on their status as facultative or obligate insect pathogens. In addition, they all have the identical ability to generate a wide range of virulence factors to control the insect immune system and the host microbiome (Boemare and Tailliez, 2009). The bacterial symbionts of EPNs have piqued the interest of researchers because they play a pivotal role in insect pathogenicity as well as the evolution of their hosts, and they are employed in biological control (Boemare et al., 1997). These symbiotic organisms and their nematode hosts have a high degree of specificity. Historically, their mutualistic relationships with the genera *Steinernema* and *Heterorhabditis*, respectively, have attracted much attention to the bacteria *Photorhabdus* and *Xenorhabdus* (Ciche et al., 2006). Their distinct associations with two divergent hosts, nematodes and insects, have dominated the study of these two bacterial taxa. The nematode has been considered to be a critical agent for transmitting their symbionts into desired insect hemocoel. Despite their sensitivity to ultraviolet rays and desiccation in the field, nematodes can regulate soil-inhabiting insect pests or insect pests infecting protected plants (Tsai et al., 2008). These bacteria keep living in the receptacle in the nematode intestine.

3.1. *Xenorhabdus and Photorhabdus: history and taxonomy*

Several research groups have focused on studying *Xenorhabdus* and *Photorhabdus* bacterial species, with the main goal of using them in agronomic, medical, and industrial applications (Bhat et al., 2017a; Fischer-Le Saux et al., 1999a). Species belonging to *Xenorhabdus* and *Photorhabdus* are all non-spore-forming rods, Gram-negative and optionally anaerobic bacteria from the Enterobacteriaceae family. Recently, with the identification of 30 *Photorhabdus* species/subspecies and 33 *Xenorhabdus* species/subspecies, the classification process of multiple microsymbionts carried by entomopathogenic nematodes has been extensively reevaluated (Loulou et al., 2023; Machado et al., 2023; Ritter et al., 2023). In general, these two genera possess an indistinguishable life style and close taxonomic origin, unlike their EPN hosts, *Steinernema* and *Heterorhabditis*, which are classified into two different

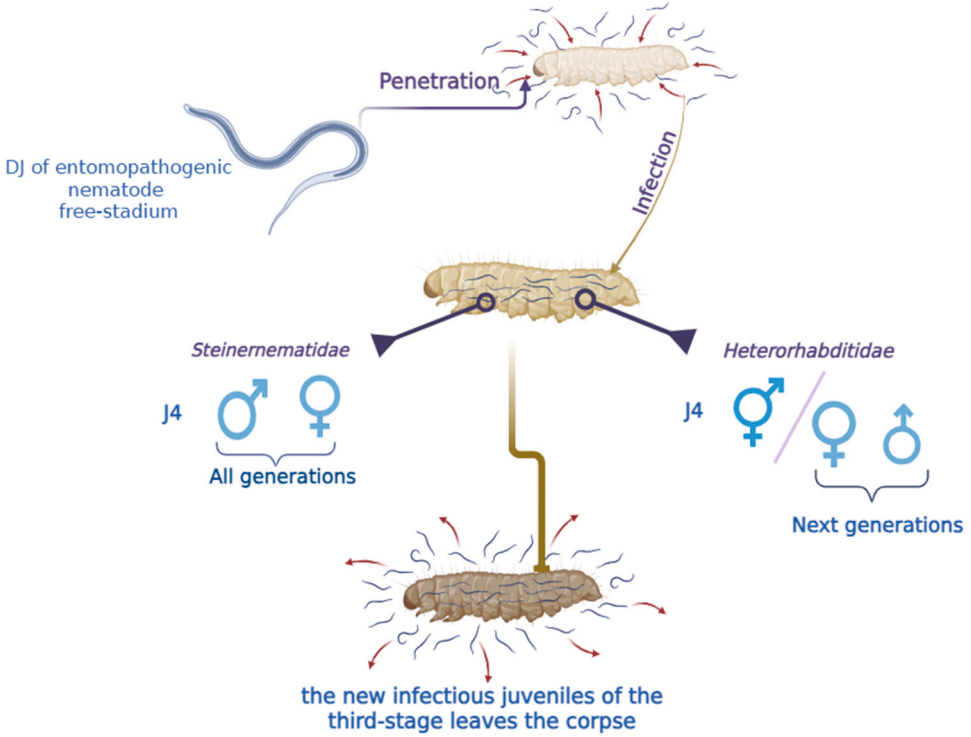

Fig. 3. Scheme illustrates the life cycle of entomopathogenic nematodes.

clades (Martens and Goodrich-Blair, 2005). *Photorhabdus* and *Xenorhabdus* species are well recognized to compose the major part of symbiotic bacteria of entomopathogenic nematodes, from the families Steinernematidae and Heterorhabditidae (Ferreira et al., 2013; Tarasco et al., 2011). According to phylogenetic studies, *Xenorhabdus* and *Photorhabdus* bacteria form close sister clusters, with Proteus being their closest neighbor. Based on available information, a common ancestor of these bacteria was a symbiont of both *Steinernema* and *Heterorhabditis* between 200 and 500 million years ago. However, two separate genera of bacteria with host-specific associations have evolved as a result of the selective pressure to maintain a symbiotic relationship with the nematode host (Chaston et al., 2011). In addition, studies have shown a general trend in EPN bacterial phylogenies toward increased virulence, which reflects a developmental trade-off between virulence and the ability to produce bacteriocin (Blackburn et al., 2016). Nevertheless, there are some notable differences, such as the nematode host groups, symbiotic methods used to inhabit their hosts, as well as a variety of insecticidal toxins and antibiotics produced (Sajnaga and Kazimierczak, 2020)..

In 1965, Poinar and Thomas described symbiotic bacteria isolated from the nematode *S. carpocapse* for the first time (Poinar and Thomas, 1965). It is thought that in the mid-Paleozoic era, the ancestor of Heterorhabditidae and Steinernmatidae began to investigate their mutualistic interplay with members of the Enterobacteriaceae family (Machado et al., 2021). They were initially named *Achromobacter nematophilus*, but no longer, they were subsequently transferred to the genus *Xenorhabdus*, later named *Xenorhabdus nematophilus*, and at last retitled *X. nematophila*, this change was proposed to adhere to the grammar rules of the Latin language and introduced in the scientific literature from 2000 (Burnell and Stock, 2000; Wee et al., 2000). As a result, until 1993 the genus *Xenorhabdus* was only composed of two bacterial species, notably, *Xenorhabdus luminesces* and *X. nematophila*, which were symbiotic organisms of *Heterorhabditis* and *Steinernema* nematodes, respectively (Akhurst and Boemare, 1988; Farmer et al., 1989). Based on genotypic and phenotypic features, *X. luminescens* was clearly distinguished from the other species of *Xenorhabdus* genera (Akhurst, 1988), implying the emergence of a novel genus *Photorhabdus* which interacts mutually with *Heterorhabditis* nematodes (Boemare et al., 1993). The previous classification of *Xenorhabdus* and *Photorhabdus* into distinct genera was based primarily on their morphological characters along with the pathway of mutualistic displayed by them (Thomas and Poinar, 1979). Molecular genetic methods such as DNA-DNA hybridization and sequence analysis of the 16 S rRNA gene are then used to continue this multiphase approach. The genus *Photorhabdus* was established in 1993, subsequently, bacteria of the unique species *P. luminescens* were split into the following two categories, *Heterorhabditis* nematode symbionts, and human specimens (Akhurst et al., 1996). Since then, numerous novel species with the status of subspecies have been created as a result of the stricter taxonomic system for this genus (An and Grewal, 2016; Sajnaga and Kazimierczak, 2020). This may be due to the greater variety of current species in the *Steinernema* genus (the symbiotic partner of *Xenorhabdus* species) than species of *Heterorhabditis* (the symbiotic partner of *Photorhabdus* species) (Shapiro-Ilan et al., 2019). To be sure, there are further unidentifiable species associated with both *Xenorhabdus* and their *Steinernema* partner that are known or are at least on their way to becoming identified. Therefore, in regard, the number of their close relatives, *Photorhabdus* species, has substantially multiplied during the past few years (Abd-elgawad, 2021) (Table 2).

Table 1
Current list of *Xenorhabdus* species distribution and their nematode partners from *Steinernema* genus.

Endosymbiotic bacteria	Nematode species	Country	Reference
Xenorhabdus ehlersii	*S. longicaudum*	China	Lengyel et al. (2005)
X. cabanillasii	*S. riobrave*	USA	Tailliez et al. (2006)
X. indica	*S. yirgalemense*	Ethiopia	Tamiru et al. (n.d.)
X. miraniensis	*Steinernema* spp.	Australia	Tailliez et al. (2006)
X. bovienii	*S. tbilisiensis*	Georgia	Gorgadze et al. (2015)
X. bovienii	*S. silvaticum*	Germany	Akhurst and Boemare (1988)
X. doucetiae	*S. diaprepesi*	Florida	Tailliez et al. (2006)
X. bovienii	*S. litorale*	Turkey	Özdemir et al. (2020)
X. mauleonii	*Steinernema* spp.	USA	Tailliez et al. (2006)
X. bovienii	*S. intermedium*	USA	Akhurst (1983)
X. stockiae	*S. siamkayai*	Thailand	Ardpairin et al. (2020)
X. japonica	*S. kushidai*	Japan	Nishimura et al. (1994); Machado et al. (2023); Ritter et al. (2023)
X. vietnamensis,	*S. sangi*	Vietnam	Kämpfer et al. (2017)
X. thuongxuanensis	*S. sangi*	Vietnam	Kämpfer et al. (2017)
X. szentirmaii	*S. costaricense*	Costa Rica	Lengyel et al. (2005)
Xenorhabdus sp.	*S. monticolum*	Korea	Kang et al. (2005)
X. poinarii	*S. glaseri*	USA	Akhurst (1983)
X. kozodoii	*S. vulcanicum*	Italy	Mirella (2011)
X. koppenhoeferii	*S. scarabaei*	USA	Tailliez et al. (2006)
X. romanii	*S. puertoricense*	Puerto Rico	Tailliez et al. (2006)
X. khoisanae	*S. jeffreyense*	South Africa	Dreyer et al. (2018)
X. bovienii	*S. poinari*	Czech Republic	Sajnaga et al. (2018)
X. ishibashii	*S. aciari*	China	Kuwata et al. (2013)
X. bovienii	*S. feltiae*	Denmark	Ehlers et al. (2011)
X. khoisanae	*S. fabii*	South Africa	Abate et al. (2018)
X. bovienii	*S. citrae*	South Africa	Malan et al. (2011)
X. budapestensis	*S. ceratophorum*	China	Yang (2019)
X. kozodoii	*S. boemarei*	France	Tailliez et al. (2006)
X. griffiniae	*S. litchii*	South Africa	Dreyer et al. (2018)
X. poinarii	*S. khuongi*	Florida	Digennaro (2021)
X. budapestensis	*S.bicornutum*	Serbia	Lengyel et al. (2005)
X. khoisanae	*S. sacchari*	South Africa	Dreyer et al. (2018)
X. kozodoii	*S. arenarium*	Russia	Tailliez et al. (2006)
X. khoisanae	*S.beitlechemi*	South Africa	Cimen et al. (n.d.)
X. indica	*S. abbasi*	Oman	Tsai et al. (2008)
X. szentirmaii	*S. rarum*	Argentina	Lengyel et al. (2005)
X. stockiae	*S. surkhetense*	Nepal	Bhat et al. (2017b)
X. poinarii	*S. cubanum*	Cuba	Fischer-Le Saux et al. (1999a)
X. nematophila	*S.carpocapsae*	Czechoslovakia	Martens and Goodrich-Blair (2005)
X. innexi	*S. scapterisci*	USA	Kim et al. (2017)
X.indica	*S. biddulphi*	South Africa	Cimen et al. (n.d.)
X. magdalenensis	*S. australe*	Chile	Tailliez et al. (2012)
Xenorhabdus spp.	*S. kraussei*	Germany	Akhurst (1983)
X. eapokensis	*S. eapokense*	Vietnam	Kämpfer et al. (2017)
X. khoisanae	*S. khoisanae*	South Africa	Ferreira et al. (2013)
X. hominickii	*S. karii*	Kenya	Tailliez et al. (2006)
X. bovienii	*S. ichnusae*	Italy	Tarasco et al. (2011)
X. griffiniae	*S. hermaphroditum*	Indonesia	Tailliez et al. (2006)

3.2. Potential effects as biocontrol agents and secreted compounds

Researchers have recently become very interested in entomopathogenic symbionts and their bioactive compounds because of their ability to control invasive species (Cai et al., 2017). Several studies have been carried out to determine the pathogenicity of microbial cell suspensions and cell-free supernatants of species from the genera *Photorhabdus* and *Xenorhabdus* against a variety of pathogens from various orders (Chaston et al., 2011; Goodrich-Blair and Clarke, 2007; Lacey et al., 2015). The focus on examining these bacteria is explained by several findings that have been reported in the literature. Generally, besides the abilities of these bacteria to promote reproduction and growth of the nematodes, they were shown to possess genes encoding secondary toxins with low molecular weight, such as antibacterial, antiparasitic, antifungal, and

Table 2
Current list of *Photorhabdus* species distribution and their nematode partners from *Heterorhabditis* genus.

Endosymbiotic bacteria	Nematode species	Country	References
Photorhabdus kleinii	*H. georgiana*	Georgia	Machado et al. (2018a)
P. khanii	*H. bacteriophora*	Australia	Tailliez et al. (2010)
P. stackebrandtii	*H. georgiana*	Georgia	Machado et al. (2018a)
P. luminescens subsp. *luminescens*	*H. floridensis*	Florida	Blackburn et al. (2016)
P. cinerea	*H. megidis*	USA	Machado et al. (2018a)
P. australis	*H. indica*	Australia	Machado et al. (2018); Machado et al. (2021)
P. cinerea	*H. downesi*	Ireland	Machado et al. (2018a)
P. aegyptia	*H. indica*	India	Machado et al. (2021); Orozco et al. (2013)
P. namnaonensis	*H. baujardi*	Thailand	Machado et al. (2021)
P. luminescens	*H.baujardi*	Vietnam	Glaeser et al. (2017)
P. laumondii subsp. *laumondii*	*H. safricana*	South Africa	Glaeser et al. (2017); Machado et al. (2018a)
P. akhurstii subsp. *akhurstii;*	*H. indica*	India	Geldenhuys et al. (2016); Machado et al. (2021)
P. luminescens subsp. *mexicana*	*H. maxicana*	Mexico	Machado et al. (2021)
P. temperata	*H.downesi*	Ireland	Machado et al. (2019)
P. asymbiotica	*H. indica*	Australia	Machado et al. (2018); Machado et al. (2021)
P. luminescens subsp. *luminescens*	*H. bacteriophora*	Australia	Machado et al. (2018a)
P. temperata	*H. megidis*	USA	Machado et al. (2018a)
P. heterorhabditis subsp. *heterorhabditis*	*H. zealandica*	New Zealand	Tailliez et al. (2010)
P. laumondii subsp. *laumondii*	*H. bacteriophora*	Australia	Machado et al. (2018a)
P. thracensis	*H. bacteriophora*	Australia	Blackburn et al. (2016); Machado et al. (2018a)
P. australis subsp. *thailandensis*	*H. indica*	Australia	Machado et al. (2018); Machado et al. (2021)
P. noenieputensis	*H. noenieputensis*	South Africa	Machado et al. (2018a)
P. asymbiotica	*H. indica*	India	Machado et al. (2018); Machado et al. (2021)
P. caribbeanensis	*H. bacteriophora*	Australia	Orozco et al. (2013)
P. tasmanensis	*H. marelatus*	USA	Machado et al. (2021)
P. khanii subsp. guanajuatensis	*H. atacamensis*	Chile	Glaeser et al. (2017)
P. kayaii	*H. bacteriophora*	Australia	Glaeser et al. (2017); Machado et al. (2018a)
P. hainanensis	*Undescribed* spp.	China	Geldenhuys et al. (2016)
P. bodei	*H. beicherriana*	China	Machado et al. (2021)
P. australis subsp. *australis*	*H. indica*	Australia	Machado et al. (2019); Machado et al. (2021)
P. akhurstii subsp. *akhurstii*	*H. georgiana*	Georgia	Machado et al. (2018a)

insecticide activities (Bode, 2009; Kim et al., 2017). During the complicated process of life, these bacteria are required to defeat the host by utilizing various protein toxins and also, they must destroy plenty of other microorganisms that are the main food competitor. In the first stage, mutualistic bacteria conquer the host's immune system by secreting a huge spectrum of biologically active substances. In addition, these active substances produced by *Photorhabdus* and *Xenorhabdus* are pivotal in the bioconversion of host insects and in protecting the host cadaver from competitors (Thomas and Poinar, 1979), besides their inhibitory potential against pest damage in short periods to provide an eco-friendly control (Koppenhöfer et al., 2020). To stay competitive for nutritional resources, *Xenorhabdus* and *Photorhabdus* generate phage-derived antimicrobial substances, proteins, insect toxin complexes, and numerous bioactive components (Bode, 2009). Besides their symbiosis *Steinernema*, the ability of *Xenorhabdus* bacteria to survive in fresh soil and water for six days has undoubtedly opened up a new avenue with a specific timeframe for their potential biological control applications (Morgan et al., 1997). Different strains of *Xenorhabdus* produce secondary metabolites with a broad array of bioactive components, including insecticidal, antibacterial, nematocidal, antifungal, and cytotoxic properties (Abd-Elgawad, 2022). Paul et al. (1981) discovered multiple novel antimicrobial properties produced by *Xenorhabdus* spp. Several more metabolites of *Xenorhabdus* have been found since this discovery. In another study, they described potent antimicrobial peptides produced by *Xenorhabdus szentirmaii* and *X. budapestensis*, putting more emphasis on their impact on the following plant pathogens*; Dyckeya, Burkholderia, Clavibacter, Agrobacterium, Curtobacterium, Erwinia* and many others bacteria (Fodor et al., 2012). However, the latest transcriptomic analysis of the mutualistic relation of *S. puntauvense-X. bovienii* and *S. carpocapsae-X. nematophila*, revealed substantial metabolic shifts related to raising conditions, in addition to bacterial interactions. Substances that have been identified in previous research, include small molecules such as benzylideneacetone (Ji et al., 2004), phenethylamines, and indole iodinine (Fodor et al., 2022) along with more complex molecules such as xenocoumacins (McInerney et al., 1991), xenorhabdins, and xenorxides (Park et al., 2005) (Table 1). Furthermore, the leucine-sensitive protein (lrp) in *X. nematophila* may modulate nematode symbiosis and insect infectivity (Hussa et al., 2015). Lrp may indeed have an important function in antibiotic production, while strains missing the lrp gene exhibited low antimicrobial activity or even may be absent against *B. subtilis* and *M. luteus* (Cowles et al., 2007). Plants that express specific genes of *X. nematophila* can develop immunity to certain pathogenic species. Tomato plants expressing *X. nematophila*-specific genes developed resistance against the cotton bollworm disease as well as tolerance to temperature changes and salt stress (Kumari et al., 2015). Similarly, a large number of research studies have been conducted and are still being conducted to assess the efficacy of entomopathogenic bacteria against pathogenic fungi. In this sense*, X. nematophila* has been found to excrete cycle lipopeptide with a lysin-rich residue that seems to be incredibly beneficial against fungal species, for both animal and plant pathogens (Gualtieri et al., 2009). Moreover, toxins (Tcs) excreted by *Xenorhabdus* isolates stimulate immunosuppression in insects by blocking eicosanoid production (Dunphy and Webster, 1984; Park and Kim, 2000). Approximately 8 to 10 silencer metabolites of insect resistance are generated by *X. nematophila* (Eom et al., 2014). Also, *X. budapestensis* was proven to generate fabclavins, which are antibiotic and insecticide combination compounds (Akhurst, 1982; Sergeant et al., 2006). Despite mutualistic nematode absence, *Xenorhabdus* species have always been toxic to insects, with the ability to destroy them after experimental inoculation. Hence, various research have been carried out to utilize species of *Xenorhabdus* for pest management, due to their potent insecticidal activity (Moth et al., 2021). All examined strains of *X. nematophila* screened positive for insecticidal properties against invasive species and against different insect orders namely, the mosquito larva *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae), the mustard beetle *Phaedon cochleariae* (Fabricius, 1792) (Coleoptera: Chrysomeloidea), and the cabbage white caterpillar *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera: Pieridae) (Sergeant et al., 2006). Moreover, *X. nematophila* HB310 outperformed the other nine bacterial symbionts of EPNs in terms of insecticidal activity against Locusta *Migratoria manilensis* (Meyen, 1835) (Orthoptera: Acrididae) (Gaugler, 2004). Several *Xenorhabdus* species seem to have greater efficacy against pests and diseases than others belonging to the above-mentioned genus. Adult *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae) demonstrated resistance to *X. innexi* (Sicard et al., 2003), which was also inefficient in controlling *Manduca sexta*

(Linnaeus, 1763) (Lepidoptera: Sphingidae) larvae, whereas *X. nematophila* was impactful against both insect species (Kim et al., 2017). Moreover, the cell-free culture of the strain DSM 16342 of *X. budapestensis* has been found to stimulate more than 50% of *Ectomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae) suppression, revealing the presence of a powerful insecticidal compound excreted by this strain. After that, they highly suggested this strain as a good option for a biocontrol agent against *E. ceratoniae* attacking *Punica granatum* fruit tree species (Alotaibi et al., 2021). Also, *Xenorhabdus* species could lead to a high suppression rate of *S. exigua* in their third-stage larvae, in contrast, they showed weak pathogenicity in the fifth-stage larvae of *S. exigua* (Nguyen & Smart, 1990). In another study, they hypothesized that the high mortality in the third larval stage was caused by antibacterial properties against *B. cereus*, an intestinal symbiont required for *S. exigua* progression. Therefore, this bacterium must be liberated inside the hemocoel to increase the pathogenic potential of *Xenorhabdus* species in the fifth larval phase (Jung and Kim, 2006). Similarly, when *X. innexi* was infused into many insect species, it showed no insect infectivity; however, when cell cultures were used, some strains exhibited larvicide activity *against Aedes, Culex,* and *Anopheles*. The Xlt component, obtained from this microbial pathogen has been characterized as a low molecular weight lipopeptide with toxic behavior against mosquito larvae. the structure of this protein contains a high concentration of amino acids including asparagine, diaminobutyric acid, histidine, glycine, and serine. And the portion lipid contains at least one oxo-fatty acid (C8 - C20) (Sarazyn, 2014).

The substances identified in previous research include small molecules such as benzyl derivatives, which are known to possess insecticidal activity, and others like xenocoumacin I and II, photopyrones, and toxoflavin, which are known to have broad-spectrum antibiotic activity. In addition, *X. nematophila* has also been shown to secrete a range of factors, including the enterotoxin complex (encoded by genes encoding xptABC), the Pir protein, and an 88-kDa protein that elongates the lifespan of *C. elegans* (Moth et al., 2021). In addition, Sifuentes-Rincón et al. (Sergeant et al., 2006) discovered that *X. nematophila* can enhance the survival of the honeybee *Apis mellifera* (Linnaeus, 1758) (Hymenoptera: Apidae) infected with the parasite *Nosema ceranae*. This study indicated that *X. nematophila* could be a potential biocontrol agent for *N. ceranae*, which is a serious threat to honeybee populations.

Similar to the above-mentioned genus, species belonging to the *Photorhabdus* genus could also serve as a promising option for broadening the biocontrol of several plant pathogens and pests; due to their rich metabolism from diverse arrays of beneficial bioactive molecules (Ahuja et al., 2021; Da Silva et al., 2020). This theory is founded on an abundance collection of *Photorhabdus* spp. that encodes for the production of pertinent molecules including enzymes, bacteriocins, antibiotics, and toxins. Further to that, new *Photorhabdus* species were found, with additional genes encoding desirable characteristics (Muangpat et al., 2020; Xiao and Wu, 2019). As a direct consequence, broad biological control action is feasible against bacteria, fungi, mites, oomycetes, and insects ravaging plants and to a limited extent, animals. These compounds have been reported to have antiparasitic, fungicidal, antibiotic, and insecticidal properties (Tobias et al., 2017). They are beneficial, for example, against the cotton mites *Aphis gossypii* (Glover, 1877) (Hemiptera: Aphididae) and *Tetranychus* (Baker & Pritchard, 1960) (Acari: Tetranychidae) (Kulkarni et al., 2017), antibiotic-resistant bacteria (Williams et al., 2005) *Colletotrichum gloeosporioides* (causal agent of anthracnose in strawberry) (Tu et al., 2022), oomycetes that can effectively restrict profitability of fruit trees (Shapiro-Ilan et al., 2009), and sometimes even vector insects of diseases in humans, especially the mosquito species *A. aegypti* (Da Silva et al., 2020). Also, *Photorhabdus* spp. the cell-free filtrate possesses insect-suppressing properties. For instance, the *P. laumondii* (TT01 strain) genome was demonstrated to encode for a broad selection of metabolic molecules, namely toxins, proteases, lipases, hemolysins, and adhesins, as well as several antibiotic substances (Zamora-Lagos et al., 2018). Extracts of this strain were identified as harmful to *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) (Shrestha et al., 2012), and filtrates of *P. luminescence* were evaluated for their efficacy against *Helicoverpa zea* (Body, 1850) (Lepidoptera: Noctuidae). Other compounds comprise genistin, stilbene derivatives, and anthraquinone extracts, all of which are extremely harmful to insects (Chalabaev et al., 2008). Extracted derivates of Anthraquinone from *P. temperata* were proven to have a toxic effect on several mosquito species (Ahn et al., 2013).

Interestingly, recent studies have suggested that *Xenorhabdus* and *Photorhabdus* bacteria can produce compounds with potential pharmaceutical applications. For instance, Gaugler (2004), identified a novel antimicrobial peptide produced by *X. nematophila* that exhibited activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Similarly, Sicard et al. (2003) discovered a new antibiotic compound, nematophin, produced by *X. nematophila* that was active against a variety of Gram-positive and Gram-negative bacteria. Furthermore, Alotaibi et al. (2021) reported that *Photorhabdus asymbiotica* produced a novel antibiotic, asymbioccin A, that was effective against both MRSA and VRE.

4. Complex nematode-endosymbiotic bacteria

The pathogenicity process of the EPNs-Bacteria complex remains mysterious, sparking the interest of numerous studies aimed at elucidating the intricate interactions among nematodes, bacteria, and host insects. The objective of these research endeavors is to gain a better understanding of the insecticidal potential of this complex and to optimize its use in biological control programs (Dillman et al., 2012; Hazir et al., 2003, 2004). The life cycle of the nematode/bacteria complex encompasses a common phase during which both organisms cooperate to enhance their pathogenic capability. During this phase, several crucial steps unfold: host-seeking, host penetration, mechanisms to escape and overcome the host's immune barriers, host infection, and the resumption of the cycle (Dillman et al., 2012; Hazir et al., 2003, 2004). DJs endeavor to instigate infection within the target host; however, they initially undergo a comprehensive process to achieve infection, commencing with the search for a suitable host.

4.1. Host Search and Recognition Strategies

The search is not a random process it unfolds based on several criteria depending on the strategy adopted by the parasite. The quest for the host can be categorized into two main approaches. The first is based on various encountered stimuli that require a response from the parasite, whereas the second relies on the parasite's mode of movement within its environment. The latter comprises two types: cruise and ambush nematodes.

Ambush nematodes are stationary and target mobile insects near the soil surface, associating with their hosts through a process called "nictation". Cruise nematodes, on the other hand, are highly mobile within the soil and target sedentary hosts at varying depths in the soil. Consequently, EPNs exhibit three distinct behaviors to enhance and facilitate host detection (Lortkipanidze et al., 2016). These mechanisms are based on three modes in EPNs:

Crawling Mode: this mode involves the movement of EPNs through sinusoidal movements in the soil, using surface forces generated by water to help them move horizontally (forward and backward). This mode is generally adopted by cruise-type EPNs (Askary et al., 2018; Lortkipanidze et al., 2016; Wallace and Croll, 1971).

Standing Mode: this mode varies between species. Some species raise the anterior part of their bodies and begin moving it back and forth, whereas others lift almost their entire bodies and initiate a swaying motion by leaning on their tails (Campbell and Gaugler, 1993; Campbell and Kaya, 2000; Lewis et al., 2006). This behavior is commonly observed in *Steinernema* (Campbell and Kaya, 2002).

Jumping Mode: during this mode, the EPN forms a loop with its body

to propel itself into the air (Campbell and Gaugler, 1993; Campbell and Kaya, 2000).

Furthermore, entomopathogenic nematodes rely on various environmental signals to recognize and locate their hosts. Host recognition in nematodes is facilitated by their response to chemical stimuli or organic matter (such as fecal material) and by detecting the cuticles of the pest. In addition, they can be attracted by volatile metabolites secreted by the insect, such as the released CO2 (Dillman, Guillermin, et al., 2012; Van Tol et al., 2001).

In this context, a study conducted by Van Tol et al. (2001), revealed that the *Thuja occidentalis* plant, infected by *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), emitted signals indicating the presence of the parasitic insect through the release of chemical stimuli. These signals attracted the infective juveniles (IJ) of *Heterorhabditis megidis* toward *O. sulcatus*, leading to the initiation of infection (Van Tol et al., 2001).

On the other hand, Lewis and colleagues (1993), found that *Steinernema glaseri* responds to the volatile signals of a host insect in the presence of CO2, and they demonstrated that the positive response of *S. glaseri* is constrained in the absence of CO2 (Lewis et al., 1993).

4.2. Penetration Inside the Host

Once EPNs are attracted by one or various stimuli, they will guide themselves toward the host insect to enter it to initiate infection. Penetrating inside an insect is not an easy step, and DJs of the EPN complex continue their assessment to avoid any repellent stimuli they may encounter at the penetration sites. Insects have various entry points, and their ability to penetrate depends on the nematode species involved (Batalla-Carrera et al., 2014; Helms et al., 2019).

The choice of penetration pathway is very important for EPNs, as an inappropriate choice can be mortally to the nematode (Table 3). The choice therefore depends on the nematode species, the target host, and the pathway used. As mentioned earlier, the insect has several entry sites such as the mouth, anus, intestinal cuticle, and tracheal system. Peters and Ehlers (1994) reported that *S. feltiae* uses the integument as the primary entry site for leather jackets. Furthermore, it was observed that *S. carpocapsae* enter sawfly preenymphs *Cephalcia lariciphila* (Wachtl, 1898) (Hymenoptera: Pamphiliidae) via anus and mouth, but spiracles are the preferred entry site (Georgis and Hague, 1981). In addition, adult arthropods, especially ticks, present the gonad openings as the main entry route for DJs (Dillman, Chaston, et al., 2012; Samish and Glazer, 1992).

After successfully entering one of the insect's most coherent pathways, the DJs find it in the host's hemocoel. The hemocoel is the site of non-self-recognition by the host's immune system (IS), and when DJs enter the hemocoel, a non-self-response is triggered. DJs face the risk of encapsulation if they are discovered by the IS (Liesch and Williamson, 2010; Wilson-Rich et al., 2007). EPNs can avoid getting encapsulated in two ways. First, by inducing multiple infections to disrupt encapsulation and actively (Yu and Kanost, 2004). Second, by suppressing the encapsulation response by the secretions of the symbiotic bacteria (Brivio et al., 2002; Mahar et al., 2005). After developing various strategies to seek out and recognize the host until they manage to penetrate the insect's interior, these events enter a stage known as the phoretic phase. During this stage, entomopathogenic nematodes engage in a mutual interaction with two partners, one of which transmits (the phorent, in this case, is bacteria) and the other serves as a carrier vector (the phoretic in this case is nematodes) (Askary and Abd-Elgawad, 2021; Koppenhöfer and Gaugler, 2009). However, the penetration of the DJ into the hemocoel induces the end of the phoretic phase and the transition of the EPNs-Bacteria complex to the pathogenic phase.

Table 3
Insect penetration routes and the obstacles that present.

Routes of entry	Risks
Mouth	1. Orifice width can eliminate EPNs (Eidt and Thurston, 1995) 2. Mandibles can be the cause of EPNs' death from crushing. (Gaugler and Molloy, 1981)
Anus	1. Orifice width can eliminate EPNs. (Eidt and Thurston, 1995) 2. EPNs can be expelled by defecation (Lewis et al., 2006)
Intestinal cuticle	1. Intestinal fluid can be toxic and an environmental barrier to EPNs. (Wang et al., 1995)
Tracheal system (ST)	1. Mechanical barriers, such as the ST's sieve plates, can eliminate invaders (Forschler and Gardner, 1991)

4.3. Pathogenic phase

We will provide an overview of the insect immune system before discussing the processes of the second phase of the complex's life cycle.

4.3.1. Overview of Insect Immunity

Insects have developed a sophisticated innate immune system to cope with microbial and parasitic attacks in their environment. This system includes physical, cellular, and biochemical defense mechanisms, as well as specific molecular recognition to detect and combat invaders (Tidbury et al., 2012; Torrecilhas et al., 2020). The innate immune system relies on the interaction between endogenous receptors and exogenous signals (Leclerc and Reichhart, 2004; Lemaitre and Hoffmann, 2007). Insect pathogen recognition receptors (PRRs) are typically localized in the membranes of immunocompetent cells or transported through body fluids. Moreover, pathogen-associated molecules (PAMPs) represent the signals released by an invasive agent (Fig. 4) (Lemaitre and Hoffmann, 2007; Péan and Dionne, 2014; Ulvila et al., 2011). PAMPs have a critical feature of structural conservation between species of a particular class. These structures include Gram-positive bacterial lipoteichoic acid, bacterial pilin and flagellin, bacterial lipopolysaccharides (LPS), sugars such as fungal beta-glucan (β-Glu), bacterial peptidoglycans (PGN), lipids, proteins, and nucleic acids, such as single- or double-stranded RNA (Cluxton et al., 2015; Medzhitov and Janeway, 2002; Panawong et al., 2022; Yu and Kanost, 2004).

4.3.1.1. Humoral immune response. Hemolymph circulating through the respiratory system is the primary site of humoral defense. It contains hemocytes that secrete and release immune factors known to help identify and eliminate pathogens (Carton and Nappi, 2001; Pila et al., 2016; Schmid-Hempel, 2009). Moreover, two primary systems, prophenoloxidase-phenoloxidase (proPO), lysozyme, and antimicrobial peptides (AMP) are all used to induce non-self-elimination (Amparyup et al., 2013; Callewaert and Michiels, 2010; Ercan and Demirci, 2016). The proPO system is an enzymatic cascade that becomes activated following interactions between humoral PRRs and non-self PAMPs. It concludes with the activation of the terminal phenoloxidase enzyme, which oxidizes phenols to quinones and self-catalyzes to form melanin (Strand, 2008; Sukumaran et al., 2019; Vidya et al., 2018). In addition, melanization which activates the proPO complex and stimulates the formation of melanin, is a rapid and effective humoral defense mechanism against parasites. This process includes creating multiple layers of melanin around the invader's body to encapsulate it (Boraschi et al., 2020; Cooper, 2018; Irazoki et al., 2019).

The lysozyme and AMP pathway involves the synthesis of antimicrobial peptides within the fat bodies and their distribution into the hemocoel (Castillo et al., 2012; Ozakman and Eleftherianos, 2021). AMPs are thermostable cationic molecules with a variable amino acid structure that demonstrate significant antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. These molecules undergo processing in response to foreign agents, which stimulate fat body cells and activate the Toll and Imd pathways. The resulting synthesis of AMPs provides an effective defense mechanism (Sun et al., 2017; Wang and Lai, 2010; Yeung et al., 2011). Lysozyme is an enzyme that can hydrolyze bacterial wall peptidoglycans, such as the

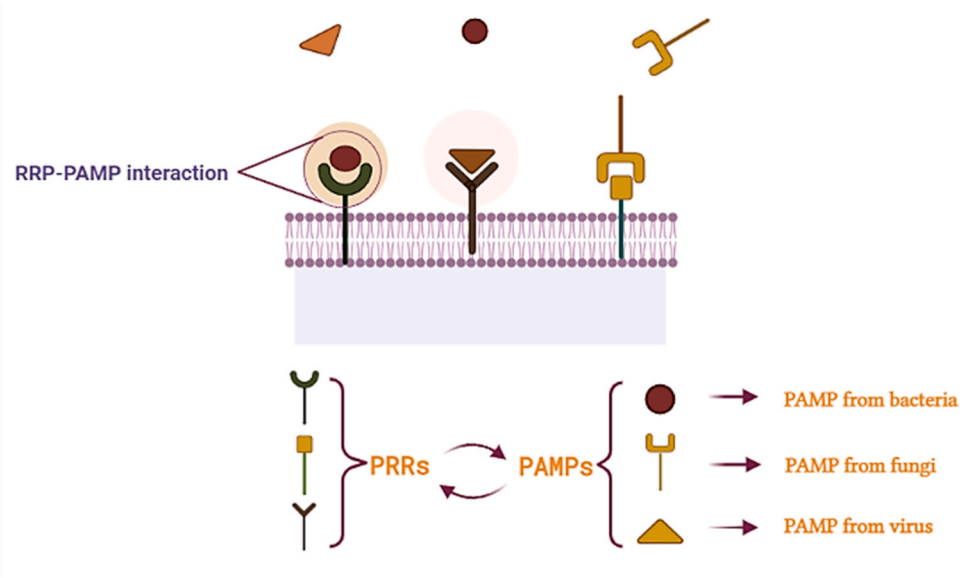

Fig. 4. Illustrative diagram of the key phase of the innate immune system. (a) interaction between RRP and PAMP; (b) diversity of PAMPs signals released depending on the pathogen.

N-acetyl-D-glucosamine residues in PGN, the major component of the Gram-positive bacterial cell wall (Eleftherianos et al., 2018).

4.3.1.2. Cellular immune response. When a foreign body that is not of the insect's kind remains in the insect's hemocoel cavity, the cellular immune defense pathway quickly becomes involved and mobilizes various cellular components of the unknown body's immune system, such as nematodes, bacteria, and fungi (King and Hillyer, 2012; López-Uribe et al., 2016). The insect's immune system rapidly recognizes and responds to foreign invaders when they enter the hemocoel. This rapid mobilization is critical to the survival of the insect, as pathogens such as entomopathogenic nematodes and their associated bacteria can invade and kill the host within a short period (Hyršl et al., 2015a). The ability of the immune system to distinguish self from non-self triggers a series of cellular responses aimed at neutralizing the threat. The cellular defense process depends on conscripted hemocytes to safeguard the host via different pathways, such as cell-mediated melanization, encapsulation, nodule formation, and phagocytosis (Dubovskiy et al., 2016; Kwadha et al., 2017; Merkling and Lambrechts, 2020; Raymond, 2019). The primary defense mechanism against protozoa and bacteria is phagocytosis. This process enables cells, namely granulocytes and plasma cells, to absorb small particles. Nevertheless, the rate of phagocytosis by hemocytes is triggered by bacterial secretions, including LPS, glucans, and PGNs (Forst and Clarke 2002a, 2002b; Murfin et al., 2012). The efficiency of this response is determined by the relationship between the organism's structure that is phagocytosed and the specific cell involved (Binda-Rossetti et al., 2016; Nurashikin-Khairuddin et al., 2022; Topalović and Vestergård, 2021; Yooyangket et al., 2018). When phagocytosis proves ineffective in absorbing invaders due to their size, encapsulation serves as a cellular defense mechanism. Hemocytes (plasmocytes and granulocytes) then activate to form multiple layers, which are covered by a layer of melanin to encapsulate the foreign body (Eleftherianos et al., 2007; Rosales, 2011). The cellular immune response has been shown to be an important component of the immune response of insects, rapidly mobilizing cellular components of the invading IS to protect the host. The cellular defense mechanism comprises diverse hemocyte types that participate in pathways such as cell-mediated melanization, encapsulation, nodule formation, and phagocytosis (Nazario-Toole and Wu, 2017; Sideri et al., 2008; Ulvila et al., 2011). The success of this response is dependent on the correlation between the structure of the phagocyted organism and the cell involved (Fig. 5). Thus, understanding the mechanisms of cellular defense in insects is crucial for the development of effective strategies for controlling insect-borne diseases (Binda-Rossetti et al., 2016; Castillo et al., 2011; Cooper and Eleftherianos, 2016; Stuart and Ezekowitz, 2008).

Hyrsl's group has made important contributions to the understanding of the immune reaction of insects, particularly against entomopathogenic roundworms and their bacterial symbionts. Her studies show that they secrete various factors that interact with their immune systems in an attempt to suppress them (Hyršl et al., 2015a). In Drosophila, a model organism for these studies, the immune response involves a sophisticated series of mechanisms. These include coagulation enzymes, recognition molecules, and eicosanoids (Hyršl et al., 2015b). Underscoring the complexity of the insect immune system, these components are critical for identifying and controlling nematobacterial infections (Hyršl, 2018).

The primary concern is whether this system can offer complete protection to insects from external aggression while preserving them. In other words, how can entomopathogenic nematodes overcome these obstacles and evade detection?

Entomopathogenic nematodes can escape immune barriers to acquire a food source and establish a niche for their development. Two strategies are used by invading parasites to evade immune defenses: immune suppression and molecular mimicry. When parasites target young hosts with low immune competence, these mechanisms are more effective (Lie et al., 1976; Pila et al., 2016; Schmid-Hempel, 2008, 2009). Molecular mimicry is a form of camouflage in which the invader either produces host-antigenic auto-proteins that are exposed on the surface of the parasite or disguises itself by sequestering host molecules to form a body shell. In this way, the parasite is able to evade the immune-competent defense system (Cusick et al., 2012; Garrouste et al., 2016; Rojas et al., 2018). Infection mechanisms of the nematode-bacteria complex have been the focus of many studies to understand how this complex infects hosts and completes its life cycle.

4.4. Infection strategies of the insect by the EPNs-Bacteria complex

In the early infection stage of the pathogenic phase, nematodes enter a mimicry mode, rendering them unidentifiable by immune response cells like hemocytes and proPO (Cooper et al., 2019; Peña et al., 2015). To overcome barriers such as the exoskeleton and reach the hemocoel of

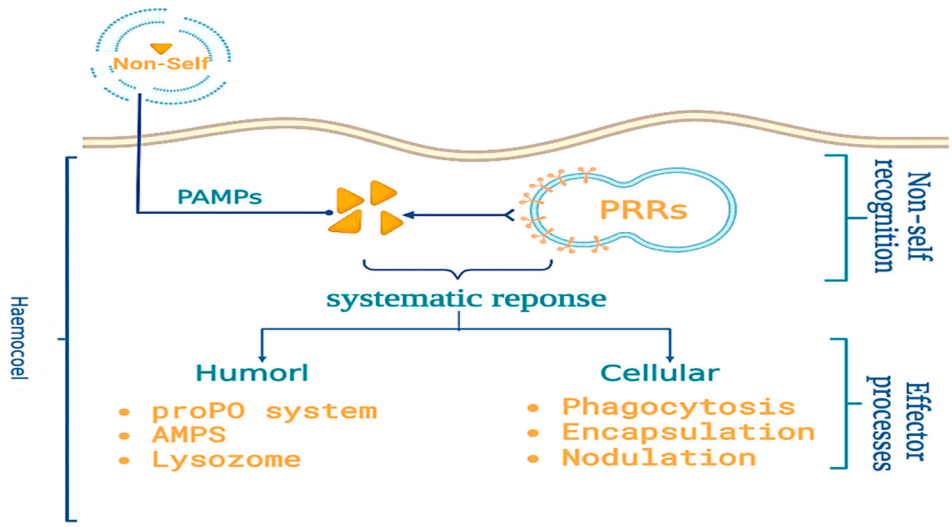

Fig. 5. Summary diagram of IS processes involved in non-self-recognition. The insect immune system. Insects have an innate IS that relies on the recognition of PAMPs that will penetrate the insect's hemocoel and PRRs. The recognition induces a system.

the host insect (Ozakman and Eleftherianos, 2021). EPNs must go unnoticed by the hemocytes. This would prevent them from encapsulating. Nonetheless, the nematode can use its body surface to interact with the immune defense systems directly (Castillo et al., 2012). Through its outer protein cuticle, which also contains small amounts of lipids and carbohydrates, this layer contains insoluble and nonstructural proteins in the epicuticular extracellular cortex in contact with hemolymph. These factors facilitate EPNs' escape from cellular encapsulation. The cuticle has a strong affinity due to hemolymph factors, and the interaction of lipids present at the epicuticle subtracts PRRs from the infected insect, contributing to the coating of the nematode's body with host components. This suggests a general immune suppression (Cooper, 2018; Debban and Dyer, 2013; Renkema and Cuthbertson, 2018).

After entering the hemocoel, the next phase begins as soon as the nematode ingests the host's hemolymph, which is seen as a signal to release these symbiotic bacteria, which in turn counteract the host's IS. However, the second part of this review reveals that the endosymbiotic bacteria of nematodes are *Xenorhabdus* spp. and *Photorhabdus* spp. These two genera survive in two phases, I and II, which differ in morphology, biochemistry, physiology, and behaviors such as antibiotic production (Banerjee et al., 2018; Dong et al., 2016; Kohli et al., 2018). Several studies have suggested that phase I bacteria may possess greater virulence than phase II bacteria, although both demonstrate potential for biological control. Therefore, the virulence of the bacteria is mainly due to their various components and factors including pili, fimbriae, and flagella that facilitate insect tissue motility and colonization, as well as LPS molecules and outer membrane vesicles that contain toxins, antibiotics, and secondary metabolites (Eleftherianos and Heryanto, 2020; Singh et al., 2014). These factors prevent bacteria from being recognized by hemocytes, resulting in complete suppression of cellular defense. This is manifested by the lysis of defense-related cells such as granulocytes and plasma cells, thus preventing processes such as phagocytosis, nodulation, and encapsulation. All of the toxins and secreted metabolites cause immune defense cells to dysfunction, rather than invade them, resulting in severe metabolic disorders that eventually cause the host's death (Nurashikin-Khairuddin et al., 2022; Topalović and Vestergård, 2021; Yooyangket et al., 2018).

Ebrahimi et al. (2011), discovered that the complete encapsulation process developed by *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) and *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) when dealing with the nematode *H. bacteriophora* takes between 2 to 4 h. Melanization, on the other hand, varies from 16 to 24 h. The formation of the hemocyte layer can take as little as two hours, implying that *H. bacteriophora* can escape their host's SI and release their bacterial load to induce secretions able to produce host death (Ebrahimi et al., 2011). Other studies suggest that the initiation of encapsulation in nematode species plays a critical role in establishing and maintaining their life cycle. In the Colorado potato beetle, *Leptinotarsa decemlineata*, the process of encapsulation begins in the center of the nematode *H. bacteriophora*, near the secretory excretory pores located near the esophageal region (Ebrahimi et al., 2011). Whereas hemocytes of *M. sexta* induce encapsulation of *Heterorhabditis* at both the head and tail ends of the nematode (Li et al., 2007). Remembering those nematodes eject their mutualistic bacteria from both the mouth and anus, this process could prove disadvantageous for the insect. As the nematode may expel bacteria well before encapsulation is complete, this could potentially negatively impact the insect's defense against other pathogens (Kenney and Eleftherianos, 2016).

Furthermore, to understand the infection potential of EPNs in insects and how they can be one of the means of biocontrol. A recent study by Benseddik et al. (2022), analyzed five EPN species isolated from different regions in Morocco. Their effectiveness was assessed in both laboratory and greenhouse experimentation, evaluating various developmental stages of *Capnodis tenebrionis*. The findings conveyed that the Moroccan EPN isolates demonstrated insecticidal properties that could serve as an alternative approach for safely and sustainably combating this destructive pest in Mediterranean countries, without causing environmental damage as traditional methods would. Indeed, in laboratory bioassays, all five EPN isolates caused significant mortality of newborn *C. tenebrionis* larvae, with mortality rates ranging from 70.5% to 98.5% after three days, and from 85.5% to 100% after five days. However, in greenhouse experiments, *C. tenebrionis* infestation of stone fruit trees was significantly reduced using all five EPN isolates, with infestation rates ranging from 6.7% to 26.7% compared with 73.3% in the control group. HBo-MOR14 strain showed the highest efficacy with corrected mortality of 100% of adult insects and only 0.2 live larvae per tree, whereas HB-MOR7 and SF-MOR9 showed lower infestation rates of 13.3%. Statistical analysis revealed significant differences in neonate mortality rates during laboratory bioassays depending on EPN isolates, humidity, and temperature, but no significant interactions were observed among these factors (Figs. 6, 7, and 8). These outcomes create exciting prospects for further exploration and future application of this research within this field (Marannino et al., 2003).

The successful survival and reproduction of EPNs inside the host, in order insect to escape the mechanical and chemical barriers of the insect, induces the transition of the nematode from the pathogenic to the

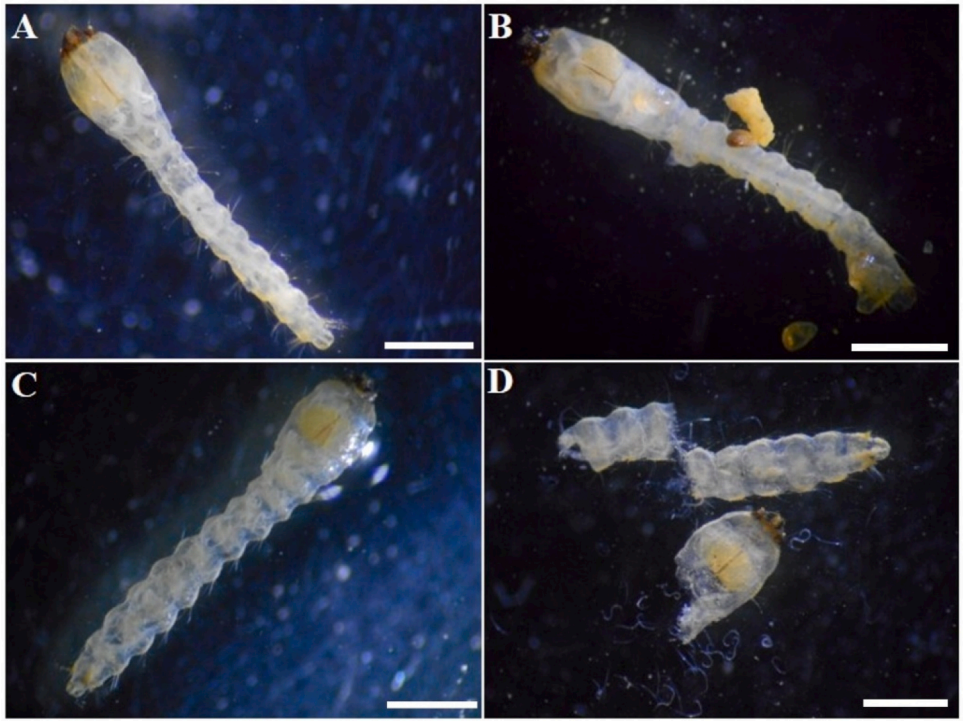

Fig. 6. Neonate larvae of *Capnodis tenebrionis* infected by entomopathogenic nematodes in Bioassay 1. A: Larva infected by *Heterorhabditis bacteriophora* HB-MOR6 (Scale bar: 1 mm); B: Larva infected by *Heterorhabditis bacteriophora* HB-MOR7 (Scale bar: 1 mm); C: Larva infected by *Steinernema feltiae* SF-MOR9 (Scale bar: 1 mm); D: Larva infected by *Heterorhabditis* sp. HJo-MOR14 (nematodes appear after dissection; Scale bar: 1 mm).

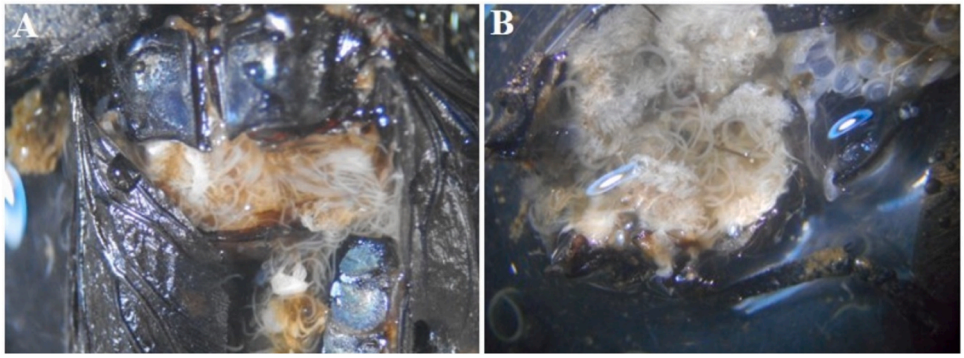

Fig. 7. Adults (females and males) of *Capnodis tenebrionis* infected by entomopathogenic nematodes in Bioassay 2. A: Picture showing nematodes belonging to *Heterorhabditis bacteriophora* HB-MOR7 inside a dissected female of *Capnodis tenebrionis* (Scale bar: 1 mm); B: Picture showing nematodes belonging to *Heterorhabditis* sp. HJo-MOR14 inside a dissected male of *C. tenebrionis* (Scale bar: 1 mm).

saprophytic. During the recuperation phase, indicating the renewal of development and feeding among infective juvenile DJs, nematodes depend on bacteria and nutrients within the host cadaver. EPNs have the ability to produce a maximum of four consecutive generations of IJs inside the host to fully utilize the existing resources. Once the new generations have matured, the IJs emerge from the host's body to retrieve their symbiotic bacteria before actively seeking out new hosts to begin the cycle anew (Koppenhöfer and Gaugler, 2009; Suwannaroj et al., 2020). .

5. Challenges and Efficacity of entomopathogenic nematodes

Against a wide range of insect pests, EPNs are considered effective biological control agents. *Steinernema* and *Heterorhabditis* are especially noteworthy because they are capable of parasitizing a large number of insect hosts. However, their effectiveness in implementation is frequently challenged due to various factors (Askary and Abd-Elgawad, 2021; Koppenhöfer et al., 2020).

5.1. Environmental factors

In order to ensure their environmental safety, the application of EPNs for biocontrol purposes requires rigorous environmental risk assessments (Marannino et al., 2010; Tarasco et al., 2015). EPNs are highly sensitive to environmental conditions including temperature, humidity, and soil type (Labaude and Griffin, 2018). Extreme temperatures can affect EPN efficiency because each EPN species has a specific temperature range for optimum efficiency (Brown and Gaugler, 1997; Labaude and Griffin, 2018). EPN movement, survival, and infectivity can also be affected by soil type, pH, and humidity (Khathwayo et al., 2021; Mokrini et al., 2020; Ramakrishnan et al., 2023). Sandy soils can facilitate the movement of EPNs but may not retain humidity very well (Deka et al., 2021; Kenney and Eleftherianos, 2016; Mokrini et al., 2020). On the other hand, Clay soils can be an obstacle to the movement of EPNs, but

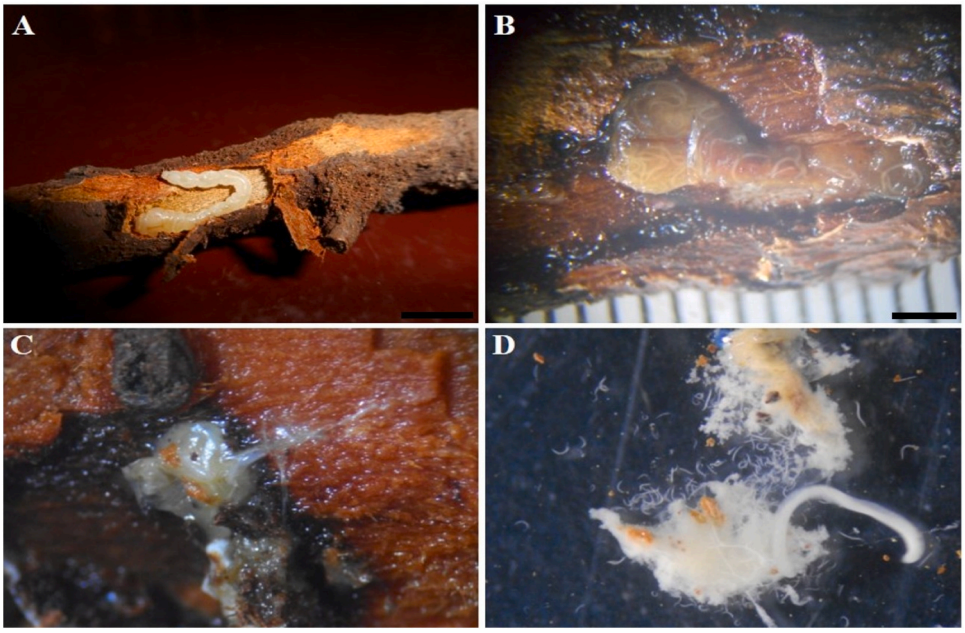

Fig. 8. Live and EPN-infected larvae of *Capnodis tenebrionis* in a pot experiment. A: Live larva in collar part of the almond tree (Scale bar: 4 mm); B: complete larva infected by *Heterorhabditis bacteriophora* HB-MOR7 inside the root system (Scale bar: 2 mm); C: Larva infected by *Steinernema feltiae* SF-MOR9 in decomposing state (Scale bar: 1 mm); D: Nematodes belonging to *Steinernema feltiae* SF-MOR9 appeared after pouring water to decomposing larva of the picture C (Scale bar: 0.4 mm).

they are a good retainer of humidity (Koppenhöfer and Fuzy, 2006). Soil pH can also affect EPN activity, with extreme pH levels being very harmful (Khathwayo et al., 2021). In this regard, Molyneux, (1985), assessed the effect of different pH levels on the survival of EPNs, and the results revealed that the pH interval of 4.5 to 6.5, is suitable for *Heterorhabditis* sp. survival, suggesting that EPNs tend to have specific pH ranges for optimal survival (Molyneux, 1985).

Furthermore, several studies underscored the effect of humidity on EPNs' reliability and movement. However, low humidity can induce desiccation and high humidity can facilitate their movement and infectivity, and keep them alive. The humidity content of the environment can determine the measure to which EPNs can penetrate the soil and reach their target pests (Kung et al., 1991; Navaneethan et al., 2010). In another study, they reported that certain EPN species, such as *Heterorhabditis* sp., and *S. feltiae*, survived longer in aerated water than in humid sand at the same temperature, highlighting the importance of moisture for their survival (Molyneux, 1985). However, Ramakrishnan et al. (2022), investigated the ability of different EPN species to be resistant to rapid desiccation (RD). They found that *S. carpocapsae* is more adaptable to RD than *S. feltiae* or *H. bacteriophora*, which require higher relative humidity to maintain their viability under RD conditions. EPN species have different levels of tolerance to desiccation. Ensuring their survival and efficacy on exposed surfaces is a major challenge, particularly in dry or arid environments (Ramakrishnan et al., 2022).

5.2. Biotic factors

The principal challenges include assessing the impact on non-target organisms, comprehension of the persistence and propagation of EPNs after application, evaluation of residues and accumulation, analysis of interactions with other control agents, assessment of the impact on biodiversity, and surveillance of the potential development of resistance in target pests (Karthik Raja et al., 2021; Lacey and Georgis, 2012). A case study by Harvey et al. (2016), presents an example of a structured approach to biotic risk assessment in relation to the control of the great pine weevil by EPNs in a forest ecosystem. The researchers applied a method of structured risk assessment to consider a range of factors, including impacts on non-target organisms and potential interactions with other control agents. The risk was quantified employing a scoring system, with a possible total score of 125 being the highest level of risk. Using EPNs resulted in a low-risk score ranging from 35 to 51, indicating a low environmental risk. This low risk was attributed to the specificity of EPNs for the target pest and the absence of adverse effects on non-target organisms and other aspects of the ecosystem. The study concluded that EPNs could be a viable and environmentally friendly option for the control of pests such as the large pine weevil in forest ecosystems, and highlighted the importance of comprehensive risk assessments before deploying EPNs, as risks may differ depending on the ecosystem and the specific species used (Harvey et al., 2016). EPNs can be used to control a wide range of insect pest species, but these pathogens may develop resistance to EPNs over time, making this biological control strategy less effective (Harvey, 2010). In a study by Campos-Herrera et al. (2015), the capacity of root-knot nematodes to develop resistance to EPNs was investigated, indicating the need for a resistance management strategy. In addition, interactions with other soil organisms are now possible, such as beneficial earthworms, which contribute to the passive movement of EPNs in soils. However, in another study, the authors showed that the mortality and physical characteristics of some EPNs can be reduced after exposure to certain earthworm species or their cutaneous excretions. This suggests that the coexistence of EPNs and earthworms may pose a challenge to EPN infectivity (Chelkha et al., 2020).

5.3. Cost-effectiveness

EPNs can be more expensive than chemical pesticides in terms of production and application, which may be a barrier to their adoption in pest management programs. Cost considerations in biological control strategies were highlighted in a cost-benefit analysis by Lacey et al. (2012), which compared the economic viability of EPNs with chemical pesticides in apple orchards (Lacey and Georgis, 2012). Supporting this, Askary and Abd-Elgawad (2021), examined the main barriers to the practical use of EPNs as biological control agents, highlighting the need to reduce costs and improve reliability to encourage their wider use (Askary and Abd-Elgawad, 2021).

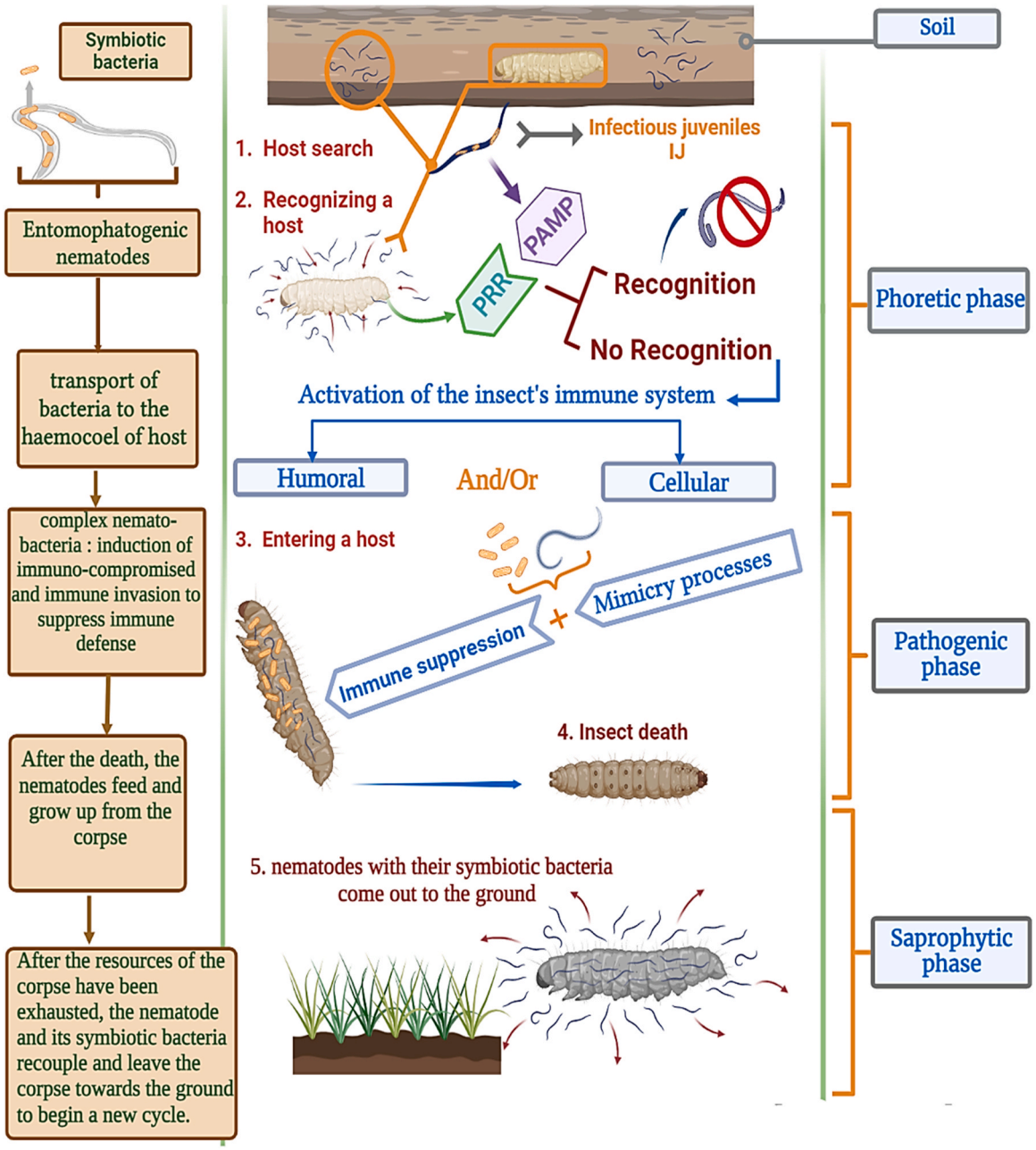

Fig. 9. Summary diagram of the infectious process of the EPNs-Bacteria complex.

5.4. *Efficacy of EPNs against various life stages of pests*

An important aspect of biological control strategies is the efficacy of EPNs against different life stages of pests. For example, Rehman and Mamoon-ur-Rashid (2022,) evaluated the infectivity of four EPN species against larvae, pupae, and adults of red palm weevils *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae). The researchers concluded that *S. carpocapsae* and *H. bacteriophora* were highly effective, causing up to 100% larval mortality in 340 h after treatment (Rehman and Mamoon-ur-Rashid, 2022). However, not all studies report such success at all life stages. A contrasting case is presented by Atwa and Hegazi (2014), who investigated the susceptibility of different life stages of the red palm weevil *Rhynocophorus ferrugineus* to 12 EPNs (Triggiani and Tarasco, 2011). While all EPN isolates were found to be pathogenic to first-stage larvae, some had a preference for larvae over pupae, with a lesser preference for adults, and others showed no preference for any stage. Field evaluations using stem injections of some EPNs resulted in significant reductions in red palm weevil populations, with efficiencies ranging from 48 to 88%, indicating a significant increase in palm survival compared to untreated controls (Atwa and Hegazi, 2014). These studies underline the need for further research to identify EPN species or strains with broad-spectrum activity against different pest life stages for more effective biological control, as well as the challenge of ensuring high mortality rates across all targeted pest life stages.

5.5. *Air parasite control*

In South Africa, a study by Platt et al. (2020), discussed the potential of EPNs for the control of surface-damaging insects and highlighted the challenge of ensuring their survival in the soil environment. EPNs have traditionally been used against terrestrial pests, but control of aerial pests is challenging due to their sensitivity to desiccation and UV light (Platt et al., 2020). It is problematic to ensure their survival and efficacy in aerial environments. As a result, they have difficulty adapting to

aerial conditions, which is critical for controlling pests such as the vine mealybug (Platt et al., 2020). However, their efficacy in such conditions can be improved through the use of adjuvants. In this regard, Platt et al. (2019), investigated the control of vine mealybug using a native nematode species, *Steinernema yirgalemense*, in combination with adjuvants. They found that the combination of Zeba and Nu-Film-P adjuvants resulted in 66% control of the pest after 48 h, compared to EPNs alone (28%) (Platt et al., 2019). EPNs have been shown to be effective against terrestrial pests. However, their use against aerial pests such as the vine mealybug has been limited by their sensitivity to desiccation and UV light. However, research suggests that the use of adjuvants can significantly improve the efficacy of EPNs in these challenging conditions.

5.6. Biocontrol of insect vectors

The efficacy of EPNs against vectors and associated pathogens is a challenge that requires further exploration. In a recent study, researchers investigated the application of EPNs as biocontrol agents against the *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae), which is a vector of the plant pathogen *Xylella fastidiosa*. This pathogen is known to cause serious diseases in a wide range of economically important plants. Even if insect vectors are effectively controlled, the challenge of preventing pathogen transmission remains. The study does not examine whether controlling meadow bugs with EPNs results in a significant reduction in *X. fastidiosa* transmission (Vicente-Díez et al., 2021).

5.7. Compatibility with chemical insecticides

The efficacy of EPNs can be influenced by their interaction with chemical insecticides. The challenge is to ensure that chemical insecticides do not adversely affect the pathogenicity of EPNs (Borgio and Susurluk, 2011). In the study conducted by Askary and Ahmad (2020), the researchers studied the pathogenicity of *H. pakistanensis* against the larvae of cabbage butterflies, *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera: Pieridae). The main objective was to determine the efficacy of *H. pakistanensis* in controlling the pest in the laboratory and the field. In fact, in the controlled environment of the laboratory, the researchers tested different levels of inoculum of *H. pakistanensis* on the 3rd and 4th instar larvae of the cabbage butterfly. They found that an inoculum level of 200 DJs was the most effective, causing significant mortality in the larvae 48 h after treatment. Moving on to real-life conditions, the researchers tested the combination of *H. pakistanensis* and a chemical insecticide, Dichlorvos 76 EC, on cabbage butterfly larvae in the field. They applied *H. pakistanensis* in combination with Dichlorvos 76 EC. The combined treatment resulted in the highest larval mortality of 79.65%, indicating a synergistic effect between EPNs and the chemical insecticide (Askary and Ahmad, 2020). The study demonstrated the challenge of ensuring compatibility between EPNs and chemical insecticides. A poor combination could potentially reduce the efficacy of EPNs or even render them useless. Achieving a synergistic effect when combining EPNs and chemical insecticides results in higher pest mortality is a challenge. This requires a thorough understanding of the interactions between EPNs and chemical insecticides.

For a wide range of insect pests, entomopathogenic nematodes hold great promise as biological control agents. Environmental conditions, soil types, and interactions with chemical insecticides affect their efficacy. While they offer a more environmentally friendly alternative to chemical pesticides, other challenges such as cost, efficacy across pest life stages, and aerial control need to be addressed. In order to realize the full potential of EPNs in pest management programmers, while ensuring a balanced interaction with the ecosystem and other control agents, further research and rigorous environmental risk assessments are essential.

6. Conclusion

In conclusion, a remarkable degree of adaptation and evolutionary coordination is evident in the strategic invasion of insect pests by EPNs and their symbiotic bacteria. Each stage is critical to the successful infection of the host insect, revealing a complex but effective mechanism of invasion and host exploitation that not only elucidates the complexity of parasitic interactions but also offers potential avenues for biological control in pest management. While the insecticidal potential of entomopathogenic nematodes is well established, there are significant differences in efficacy among species, which can be attributed to variations in the mechanisms involved in evasion, infection, and suppression of the insect immune system. Additionally, the success of the process is highly dependent on the target host and their immune system. EPNs' potential provides insecticidal activity against a wide range of pests. Their efficacy is influenced by several environmental factors such as temperature, humidity, and soil type. While EPNs provide an environmentally friendly alternative to chemical pesticides, their cost, effectiveness at different pest life stages, and interaction with chemical pesticides pose challenges. In order to realize their full potential for pest control, these challenges must be addressed through further research and rigorous environmental risk assessment.

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CRediT authorship contribution statement

Lahlali Rachid: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Mokrini Fouad:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **El Jarroudi Moussa:** Formal analysis, Investigation, Validation, Visualization, Writing – review & editing. **Joutei Abdelmalek Boutaleb:** Formal analysis, Investigation, Writing – review & editing. **Blenzar Abdelali:** Formal analysis, Investigation, Writing – review & editing. **Benseddik Youssef:** Data curation, Formal analysis, Visualization, Writing – original draft. **Kenfaoui Jihane:** Data curation, Formal analysis, Visualization, Writing – original draft. **Laasli Salah-Eddine:** Data curation, Formal analysis, Visualization, Writing – review & editing. **Goura Khadija:** Data curation, Formal analysis, Writing – original draft. **Ouijja Abderrahman:** Formal analysis, Supervision, Visualization, Writing – review & editing. **Kallali Najwa Seddiqi:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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