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ENTOMOPATHOGENIC NEMATODES AS AN ALTERNATIVE BIOLOGICAL CONTROL AGENTS AGAINST INSECT FOES OF CROPS

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ABSTRACT

Entomopathogenic nematodes belonging to the genus *Steinernema*, *Heterorhabditis* and *Neosteinernema* are the natural killers of insects belonging to different orders. These nematodes are suitable biocontrol agents as they do not possess a threat to the environment and safer to human health. Commercially entomopathogenic nematodes are exploited against insect pests of various economically valuable crops. Upon application in the field, these nematodes face many biotic and abiotic stresses which results in inconsistent efficacy in pest management. Traditionally artificial selection and hybridization techniques were adopted to improve traits related to penetration and infectivity to insect host and storage stability in the formulation. Artificially improved traits tend to losses in the external environment once the selection pressure removed. Genomics assisted breeding provides an alternative way for stable trait improvements in entomopathogenic nematodes which last for a longer period and exhibit maximum efficacy in the field against targeted insect pests. Understating their lifecycles and complex mechanisms of host's infectivity exhibited by nematode-bacterial partners would further enhance our knowledge to improve their efficacy against insect pests. In the future, there is a huge scope of developing stable commercial formulations of entomopathogenic nematodes as a suitable biological control agent.

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1 Introduction

The vices of erratic and over uses of insecticides are contaminating the aquatic and terrestrial ecosystem, posing a serious threat to biodiversity and developing resistance in insects. As an alternative, the uses of entomopathogenic nematodes are increasing as a suitable biological control agent against insect pests of economically valuable crops (Gaugler, 2018). The entomopathogenic nematodes belong to the genus *Heterorhabditis* Poinar (1975), *Steinernema* Travassos (1927), and *Neosteinerinema* Nguyen & Smart (1994) (Rhabditida: Nematoda) can kill a wide variety of insects. Among these three genus, the former two are highly exploited against insects as a biocontrol agent. The genus *Heterorhabditis* is associated with the bacteria *Photorhabdus* and genus *Steinernema* is associated with the bacteria *Xenorhabdus* (Leite et al., 2019). These nematodes perform ambushing or cruising activities (Ruan et al., 2018) to locate its hosts, and regurgitate its mutualistic bacteria once they reach inside the insect's midgut (Labaude & Griffin, 2018). The bacteria secretes multiple toxins and kill the insect by septicemia and converts the host's tissue content into a nutrient-rich medium for the growth and development of its own and its nematode

partner (Mbatia et al., 2019). These nematodes don't cause harmful effects to the environment, not allow resurgence and resistance development in insects, hence they are a suitable alternative to synthetic insecticides (Askary et al., 2018). The entomopathogenic nematodes are globally present in every continent except Antarctica (Griffin et al., 1990).

Once the infective juveniles reach inside the midgut of insects, it just takes two to three days to kill and entire lifecycles complete in between 10-15 days (Li et al., 2019). Various researches have done in the past also exhibited the suitability of these nematode's applications via traditional equipment. These nematodes also found compatible with the range of pesticides upon application in the field (Chavan et al., 2018). These nematodes are commercially produced and exploited against insects in many countries such as European nations and USA, yet their share in the pest management market is hardly 1 percent (Smart, 1995). These nematodes are produced on its hosts via in vivo or in vitro techniques on solid or liquid culture media. In the table 1, entomopathogenic nematodes and their targeted hosts are listed. The commercial share in the pest management market would only increase with the development of new production methods and improved efficiency (Saleh et al.,

Table 1 Application of Entomopathogenic nematodes against insects pest attacking different crop

Common name	Scientific name	Crop	Efficacious Nematodes
Army worm	<i>Spodoptera</i> spp.ss	Vegetables	<i>Steinernema carpocapsae</i> , <i>S. feltiae</i> , <i>S. riobrave</i>
Banana moth	<i>Opogona sachari</i>	Ornamentals	<i>Heterorhabditis bacteriophora</i> , <i>S. carpocapsae</i>
Banana root borer	<i>Cosmopolites sordidus</i>	Banana	<i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. glaseri</i>
Black cutworm	<i>Agrotis ipsilon</i>	Turf, vegetables	<i>S. carpocapsae</i>
Black vine weevil	<i>Otiorynchus sulcatus</i>	Berries, ornamentals	<i>H. bacteriophora</i> , <i>H. downesi</i> , <i>H. marelata</i> , <i>H. megidis</i> , <i>S. carpocapsae</i> , <i>S. glaseri</i>
Cat flea	<i>Ctenocephalides felis</i>	Home yard, turf	<i>S. carpocapsae</i>
Citrus root weevil	<i>Pachnaeus</i> spp	Citrus, ornamentals	<i>S. riobrave</i> , <i>H. bacteriophora</i>
Codling moth	<i>Cydia pomonella</i>	Pome fruit	<i>S. carpocapsae</i> , <i>S. feltiae</i>
Corn earworm	<i>Helicoverpa zea</i>	Vegetables	<i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. riobrave</i>
Corn rootworm	<i>Diabrotica</i> spp.	Vegetables	<i>H. bacteriophora</i> , <i>S. carpocapsae</i>
Crane fly	<i>Tipula pubera</i>	Turf	<i>S. carpocapsae</i>
Fungus gnats	<i>Lycoriella</i> spp	Mushrooms, greenhouse	<i>S. feltiae</i> , <i>H. bacteriophora</i>
Large pine weevil	<i>Hylobius abiet</i>	Forest plantings	<i>H. downesi</i> , <i>S. carpocapsae</i>
Leaf miners	<i>Liriomyza</i> spp.	Vegetables, ornamentals	<i>S. carpocapsae</i> , <i>S. feltiae</i>
Mole crickets	<i>Scapteriscus</i> spp.	Turf	<i>S. carpocapsae</i> , <i>S. riobrave</i> , <i>S. carpocapsae</i>
Scarab grubs	<i>Holotrichia</i> sp.	Turf, ornamentals	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. glaseri</i> , <i>S. scarabaei</i> , <i>H. zealandica</i>
Shore flies	<i>Scatella</i> spp.	Ornamentals	<i>S. carpocapsae</i> , <i>S. feltiae</i>
Sweet potato weevil	<i>Cylas formicarius</i>	Sweet potato	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i>

2020). These nematodes face the biotic and abiotic pressures in the field (Dzięgielewska & Skwierz, 2018), which hinder its maximum efficacy. Because there are certain traits like tolerance to cold and heat, sensation to ultraviolet light, desiccation, and persistence determines its fitness in the field (Abd-Elgawad, 2019). Traditionally artificial selection and hybridization methods had been applied in trait improvement (Lu et al., 2016). But there is a huge possibility of losing a novel improved trait once the selection pressure is removed. Nowadays genomic sequence information of entomopathogenic nematodes assisted with molecular breeding tool paving a way for the trait improvements (Sumaya, 2018). The traits improved via molecular methods would last permanently or for a longer period as compare to artificial selection and hybridization (Abd-Elgawad, 2019). Once the traits related to storage stability and host-seeking behavior would be improved, then the cost of commercial production would reduce to a certain level. Entomopathogenic nematodes kill effectively the insect belonging to most thwarting orders like Coleoptera, Lepidoptera, Hemiptera, Diptera, and Orthoptera (Belien, 2018) etc. There is a huge scope lying with the uses of these nematodes against insects in future when the eco-safety and human health is the main concern. The aim of this review is to highlight the potential of entomopathogenic nematodes as an effective biological control agent against the insect-pests of crops. The inclusion of these nematodes in integrated pest management schemes can negate the dependence on excess uses of synthetic pesticides.

2 Life cycle of EPNs

Both entomopathogenic nematodes exhibit a similar type of life cycle and mode of infection with slight differences in their formation first generation progeny. *Steinernema* undergoes continuous two sexual generations with males and females separately each time (Gauraha et al., 2018). While in *Heterorhabditis*, the first generation converts to hermaphroditic female (Zioni et al., 1992). The entomopathogenic nematodes exhibit a step by step process to infect its insect's host. Firstly they move freely in the soil to search for its host, followed by infection and penetration of its host (Alonso et al., 2018). Once inside the midgut of hosts, the nematode-bacteria exhibit a complex mechanism to defend host immunity (Eleftherianos et al., 2018). The nematode regurgitates its mutualistic bacteria inside the host's midgut and kills it by producing an array of insect-toxic protein toxin complexes (Sheets & Aktories 2016). The mechanism of killing insect host by entomopathogenic nematode is depicted in figure 1. Both nematode and bacteria undergo multiplication and produces new progeny and completes their lifecycles until the host contents limits to support further reproduction. At these stages, newly formed infective juveniles emerge out from the empty cadaver and moves freely in the soil to search for another host (Sulistyanto et al., 2018). The sequential lifecycle of entomopathogenic nematodes is depicted in figure-2.

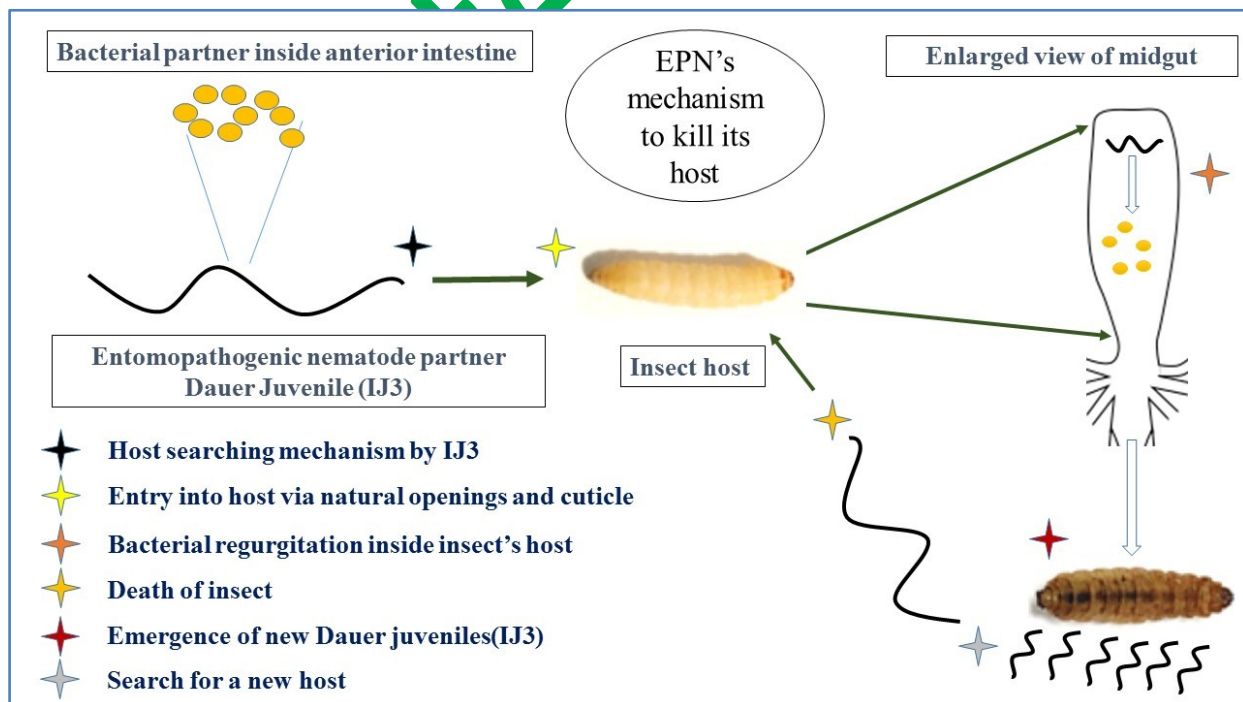
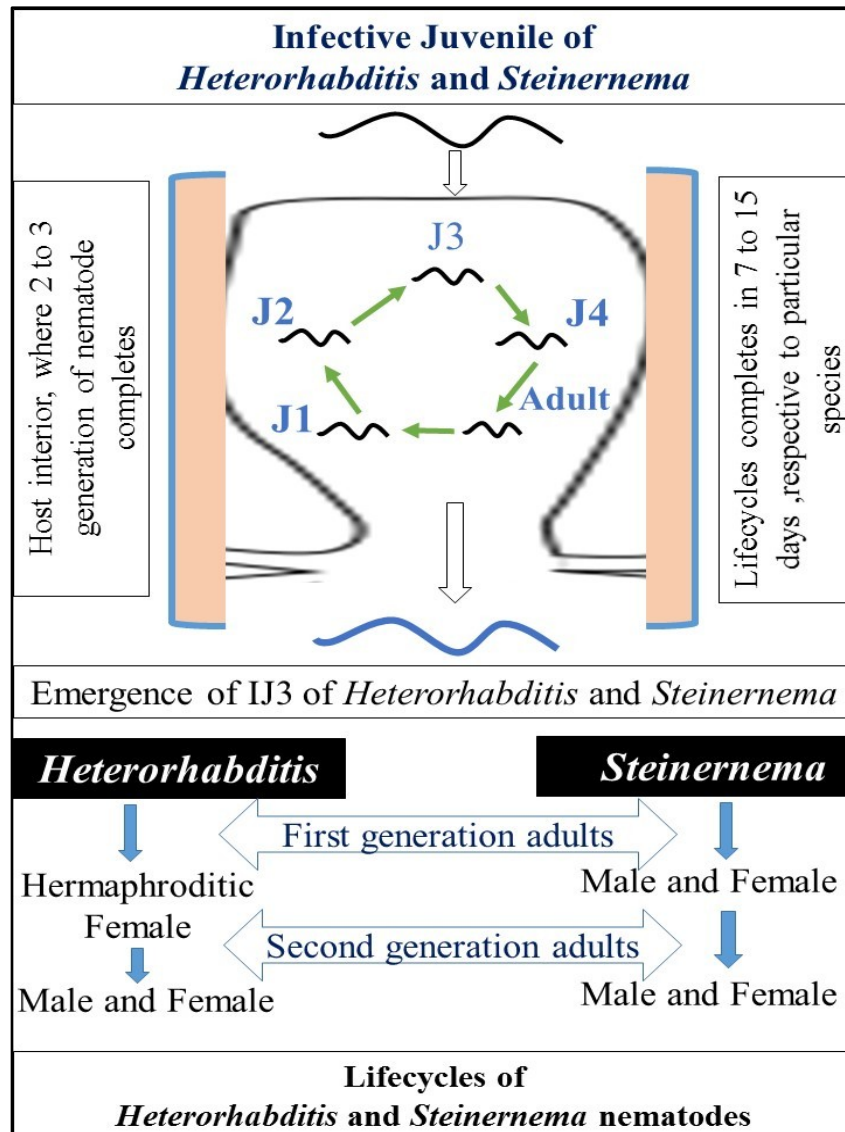


Figure 1 EPNs mechanism to kill insect host

Figure 2 Lifecycles of entomopathogenic nematodes *Heterorhabditis* and *Steinernema***3 EPN is considered as a good biopesticide**

Entomopathogenic nematodes can be used as a good biopesticides and can be use as an alternative of chemical pesticides. The following are the unique characteristics of EPN

- Ability to search (chemoreceptors) the target insect
- Quick kill of the target insect
- Broad host range
- Easily cultured
- Compatibles with many pesticides
- Easy delivery system by spraying EPN suspension or irrigation system
- Safe to vertebrates, plants and non-targets and environmentally safe
- Long-term control
- Reproductive potential (like pathogens)
- Exempt from registration requirement

4 The differences between the *Heterorhabditis* and *Steinernema* lifecycles

The entomopathogenic nematodes belongs to genus *Heterorhabditis* and *Steinernema* are proven to manage effectively insect pests of crops belonging to different orders. The lifecycles of both the EPNS i.e. *Heterorhabditis* and *Steinernema* are utmost similar with slight differentiation in their mode of entry inside the host and production of first-generation progeny (Kooliyottil et al., 2013). The entry inside the insect's host is facilitated by natural pores which include the mouth, spiracles or anus. Besides, the nematode *Heterorhabditis* can also enter via penetrating the cuticle of the insect's host as it contains a mural tooth on the dorsal wall of the buccal cavity which helps in tearing of cuticle.

5 Stages in the lifecycle

The Lifecycles of both the nematodes include a total of six stages namely egg stage, followed by four juvenile stages and adult stage. In the case of both nematodes, the third juvenile stage is the only stage that resides in the soil. The third juvenile stage is the non-feeding stage which carries the symbiotic bacteria inside their anterior part of the gut. The bacteria reside by forming a film inside the anterior gut in the case of *Heterorhabditis* nematode and a pouch-like structure in the case of *Steinernema* nematode. The third juvenile stages are also called as Dauer juvenile or infective juvenile stages (Grewal et al., 2002).

6 The role of nematode partner

In a mutualistic relationship with bacteria, primarily the nematode partner provide shelter to its bacterial partner. Nematode partner further acts as a transporting agent or vector for its bacterial partner, as bacteria can perform its effective role upon reaching inside the host midgut. Meanwhile, it protects its bacterial from the host defense processes (Gaugler, 2018).

7 The role of bacterial partner

The bacterial partner exhibits three main functions once inside the host midgut. Firstly it produces multiple toxin complexes to kill its host, followed by the production of bio-enzymes to converts host's tissue contents into a suitable growth medium for the multiplication of both the mutualistic partners. At last, bacteria encode several antibiotics compounds to inhibit the growth of secondary microbes on dead insect hosts. Additionally, the bacterial partner safeguards its nematode partner against host immune responses (Gaugler, 2018).

8 Mass production technology of Entomopathogenic nematodes

Entomopathogenic nematodes are being mass produced in several countries of North America, Europe and Asia, on both a small and

large scale, using bioreactors (Shapiro-Illan & Gaugler, 2002). They can be mass produced in two ways: (i) *in vivo* and (ii) *in vitro*. In the case of *in vivo*, insects serve as the bioreactor, whereas their *in vitro* process is carried out in artificial media (Devi & George, 2018).

This is a low technology method with low startup costs (such as a cottage industry) that involves the production of EPNs by using live insects, which are highly susceptible and easily available at a lower cost. The insects used under this method are the larvae of the greater wax moth, *Galleria mellonella*, the rice moth, *Corcyra cephalonica*, or the mealworm, *Tenebrio molitor*, which are reared in the laboratory (Griffin et al., 2005).

Generally, the last instar of *G. mellonella* is preferred, due to its high susceptibility, easy availability and high yield of IJs (Rahoo et al., 2018). The approach is based on two dimensional systems that rely on nematode production in trays and shelves (Ehlers & Shapiro-Illan, 2005). The method involves four steps: inoculation, harvest, concentration and decontamination.

8.1 Inoculation

Insects are inoculated with IJs on a tray or dish lined with filter paper or another substrate conducive to nematode infection, such as soil or plaster of Paris. The nematode dosage and host density should be optimized for maximum yield. Too low a dosage of IJs may result in low host mortality, whereas too high a dosage may result in failed infections due to competition with secondary invaders. Approximately 25–200 IJs are sufficient to cause infection on one insect larva of *G. mellonella*.

8.2 Harvest

This step is performed by using a technique based on the White trap, wherein after 2–5 days, the host insects killed by nematodes are placed above a water reservoir. The nematode produced by this method is harvested by placing moist filter paper on a concave side up watch glass surrounded with water in a large Petri dish. The progeny IJs migrate from the depleted host cadaver into the water reservoir, where they are trapped and subsequently harvested.

8.3 Concentration

IJs are decanted, transferred to a beaker and then kept in biological oxygen demand (BOD) incubator at 10–15°C. During the process, care should be taken that settling for a prolonged period may prove detrimental to the nematodes, as this often causes a lack in oxygen content. Although this may be accomplished by vacuum filtration or centrifugation for commercial *in vivo* operations, the total cost will be much higher for a centrifuge of sufficient capacity (Shapiro-Illan et al., 2004).

8.4 Decontamination

There is a chance of host material or microbial contamination on nematodes while migrating away from the cadaver. Therefore, the nematodes harvested by this method are washed repeatedly. This can be accomplished by gravity settling (Dutky et al., 1964), wherein antimicrobial compounds such as streptomycin sulfate, Hyamine® (methylbenzethonium chloride), merthiolate, NaOCl and HgCl₂ are used (Lunau et al., 1993). These compounds have not been found to have any detrimental effect on nematodes during commercial application (Shapiro-Ilan et al., 2004).

9 Availability and Importance of genomics information of entomopathogenic nematodes

Entomopathogenic nematodes exhibit a high level of pathogenic activity against insect pests of crops belonging to different orders. These nematodes have been commercialized in many countries as effective biocontrol agents against insect pests. In spite of strong pathogenic activities against insects, their full potential under insect-pest management is yet to be realized. These nematodes suffer setbacks and losses consistency when applied in the field as they suffer a direct pressure of biotic and abiotic stresses. These external stresses forces hinder their normal establishment in the field and fail to adapt to the environment. For commercial level exploitation of entomopathogenic nematodes, there is a need to improve its certain specific genetic traits. These traits include host-seeking abilities, host-penetration, and infectivity, longevity, persistence, and storage stability (Gaugler, 2018). Traditionally artificial selection and hybridization tactics were employed to improve these aforementioned traits, but once the selection pressure is mitigated, there are abundant chances of losing these traits. New modern genetics tools assisted with genomic information provide a choice for improvement of genetic traits. In recent years, at least entomopathogenic nematodes genomic sequences have been published and other sequencing projects are in progress (Yadav et al., 2015). Analysis of genomic sequences helps in identifying the candidate genes involved in specific trait

regulation. Thus these genes can be isolated and transformed in commercialized strains of entomopathogenic nematodes for better efficacy in the field. In the Table 2, the genome size and number of estimated G-protein coupled receptors (GPCRs) and proteases information are listed for different entomopathogenic nematodes.

10 Future perspectives

Entomopathogenic nematodes exhibit broad-spectrum control against insect pests belonging to different orders. Efficacy of these nematodes have been checked against various thwarting insect pests like Armyworms, plume moth, cutworms, weevils, shoot borers, codling moths, leaf miners, mole crickets, shoot flies, etc. But the results were found inconsistent as certain limiting factors affect the establishments of these nematodes directly or indirectly. The selection of a unique strain of EPNs against a target pest is the major step that decides success or failure in control aspects. None of the single strain is unique for all the key traits which include cold tolerance, heat tolerance, and desiccation, high host-seeking ability, penetration, and infectivity. Previous researches have also found that a native strain of a particular geographical region manages insect pest effectively belonging to the same geographical region. Among these to genus, *Heterorhabditis* is mostly adopted to the tropical and subtropical environment while *Steinernema* confines its better efficacy in temperate environments. Genomics insights and breeding methods provide an alternative way to improvised native strains for specific aforementioned traits. The development of commercial formulations and storage stability are the major challenging task, as these nematodes are live organisms. These nematodes have to be stabilized in the carrier material to develop a formulation and also suffer transportation shocks. There is an immediate need to search for genes responsible for longevity and persistence, as manipulation of these genes would help in the long term survival of these nematodes in commercially developed formulations. The other challenging tasks related to the entomopathogenic nematodes are the way of application in the field. These nematodes are usually applied with irrigation water, drenching in soil and spraying but

Table 2 Genome size and number of estimated G-protein coupled receptors (GPCRs) and proteases

S.No.	Nematode species	Genome size (Mb)	Estimated Putative GPCR	Estimated proteases	References
1	<i>Heterorhabditis bacteriophora</i>	77.0	82	19	Bai et al., 2013
2	<i>Steinernema carpocapsae</i>	85.6	604	268	Rougon-Cardoso et al., 2016
3	<i>Steinernema scapterisci</i>	79.4	731	357	Dillman et al., 2015
4	<i>Steinernema feltiae</i>	82.4	883	267	Dillman et al., 2015
5	<i>Steinernema glaseri</i>	92.9	806	248	Dillman et al., 2015
6	<i>Steinernema monticolum</i>	89.3	690	423	Dillman et al., 2015

the efficacy does not found the same in all the cases. In the changing scenario where the uses of synthetic pesticides are neglected, the scope of utility of entomopathogenic nematodes in insect pest's management would be promoted. In the coming future, enormous scientific studies and researches are required to understand the complex biology of these nematodes to maximize its utility under integrated pest management schemes.

Conclusion

The entomopathogenic nematodes are very effective against the insect-pests dwelling in the soil environment as these nematodes naturally thrive well in soil. These nematodes have the great potential to be used as a biocontrol agent in crop protection schemes. But the consistency of these nematodes in the field is the major challenge as these nematodes face environmental extremes once applied as formulation against a target insect-pests. In the past, many commercial formulations have been made and applied to control the insect-pests of economically valuable crops. But a broad spectrum utility of these nematodes can be achieved by trait improvements. Genomics assisted with breeding is an effective methodology to improve traits related to infectivity, persistence and storage stability of entomopathogenic nematodes. Genomics help in the identification of genes and their interaction mechanisms involved in traits regulation. The novel genes specific for a particular trait can be isolated from a donor strain and transformed in native strains of entomopathogenic nematodes for their maximum efficiency. A better understanding of the complex mechanism of entomopathogenic nematodes involved in mutualism with bacterial partner and pathogenicity to insects will enable us to enhance the utilization of these nematodes for biological control of insect pests.

Conflict Of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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