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Review Article
Entomophilic Nematodes as Biocontrol Agents Against Insect Pests: A Review

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Abstract
Entomophilic Nematodes are unique because they are the only nematodes that have evolved the ability to enter the body cavity of the insect. However, a new interest has been kindled in this tiny worm in the recent past due to its excellent potential and quick knock-down effect on insects. It is well known that entomopathogenic nematodes (EPNs) are one of the most efficient biocontrol agents of insects that pose a threat to a variety of crops. The EPNs are nematodes that are utilized extensively. These nematodes can be used to manage insect pests that infest various crops and other plants. Both *in vitro* methods, such as solid or liquid fermentation, and *in vivo* methods, like insect baiting, can produce large quantities of these advantageous EPNs. Small-scale agricultural field operations, greenhouses, or laboratories are the best environments for *in vivo* and *in vitro* EPNs generation. The EPNs are significant as environmentally friendly products because they play a critical role in managing a variety of insect pests of field crops, stored goods, and grains, etc. this study, aim to summarize the parameters influencing the *in vivo* mass synthesis of EPNs and to describe the different steps and elements involved in this process.

Key words: Entomophilic nematodes, Indian scenario, world scenario, commercial development, entomopathogenic

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INTRODUCTION

There are a few scattered references in the 17th and 18th centuries, but the 19th century, especially 30-40 years, has certainly been the most productive period for the work of mermithid nematodes, Poina[1], revealed the nematodes in Stone, Amber and Mummies. Research on biological control of insects with Entomophilic Nematodes has progressed from the 19th century with the discovery of Heydonium antiquum and *H. malutimus* work collected from Rhine lignite (Eocene) and Baltic Amber and the importance of fossils in understanding the evolution of parasites and their vectors[2].

To manage insect pests, mermithid nematodes can be widely employed in organic farming, integrated pest management, sustainable agriculture systems and biological control of insects[3].

Even though Glaser and associates cultivated enough nematodes for field testing in the 1930s, the development of efficient and reasonably priced insecticides between 1940 and 1960 stopped the usage and spread of Entomophilic Nematodes. After that, the availability of inexpensive insecticides between 1940 and 1960 hindered the use and spread of Entomophilic Nematodes (EPNs).

Following that time, when government actions in many countries caused a shortage of some of these pesticides, interest in natural pesticides, including the usage of EPNs, resurfaced[4]. A variety of hosts from all insect groups exhibit this high mortality rate[3,5].

Although there are over 40 nematode families known to control insect pests, *Agamermis unka* (Mermithidae) parasitism of nilaparvata lugens in rice fields in Korea[6]. Lepidopterans, Coleopterans, Orthopterans, Diptera and Hymenoptera are reported to be parasitized. As parasites of *Heliothis* spp., mermithid nematodes are a crop problem in India[7]. The EPNs are the best choice for creating and applying them as effective biological control agents because of their remarkable infection. Many researchers have documented some new hosts of a juvenile mermithid of the genus Hexamermis that are managed with mermithids as a biocontrol agent[8]. Poinar and Buckley[9] reported Nematode and hairworm (Nematomorpha) parasites in early Cretaceous amber.

The objective of this review is to critically assess the potential of Entomophilic Nematodes as effective biocontrol agents against various insect pests. It aims to explore their taxonomy, mode of action, ecological adaptability and compatibility with Integrated Pest Management (IPM) practices. The review also intends to highlight recent advances in their mass production, formulation and field application, while identifying challenges and future prospects for their broader use in sustainable agriculture.

Prospects for the future

World scenario: Throughout the world, there is consented effort among scientist in augmentative biological control of insects using Entomophilic Nematodes.

These nematodes were successfully applied to control agricultural and horticultural pests in many countries. The best products are available in the international market for the control of a wide spectrum of insects. Improvements in formulations have resulted in nematode products that have a longer shelf life and stability and are easier to mix and apply. The development of local markets will be a challenging task. The cost of production of Entomophilic Nematodes (EPNs) is higher when compared to insecticides and requires more training to use. Growers will be willing to pay the extra price and learn to use nematode-based products instead of an alternative chemical pesticide if only the nematode products show comparable efficacy.

Commercial development: A number of successful commercial products containing EPNs are available in the world market, namely, Skeeter Doom etc. (Fig. 1).

Nematodes as biological control agents: Mermithidae

Status: Entomophilic Nematodes occur in some 27 families in 9 major groups of nematodes, namely, rhabditoid, tylenchoid, aphelenchoid, strongyloid, oxyuroid, ascaridoid, spiruroid, fliarioid and mermithoid. These nematodes vary greatly in size and shape, have insects as intermediate or as definitive hosts and may be facultative or obligatory in their relationships with their hosts. Many species of these parasitize insects of a wide range of orders, including insect pests of agriculture, medical, veterinary and forestry importance. Nowadays, nematodes are being successfully utilised as a biocontrol agent against several insect pests and among these, mermithid nematodes are of paramount importance. Their juvenile forms occur in the body cavities of insects, usually in the abdomen and get nourishment directly from the haemolymph of the host insects. Poinar[3] works on Nematodes for the biological control of insects.

Mermithid nematodes are known to occur in 15 insect orders, *viz.* Orthoptera, Odonata, Diptera, Hymenoptera and Hemiptera. The emergence of the parasitic form of mermithids from the body cavity of the host insect produces a large hole in the chitinous exoskeleton, which results in the death of the host due to loss of body fluid. This hundred percent mortality occurs in parasitized hosts and due to this factor, mermithids are used as biocontrol agents. Some of the mermithids examples are reviewed as follows:

Fig. 1: Skeeter doom product

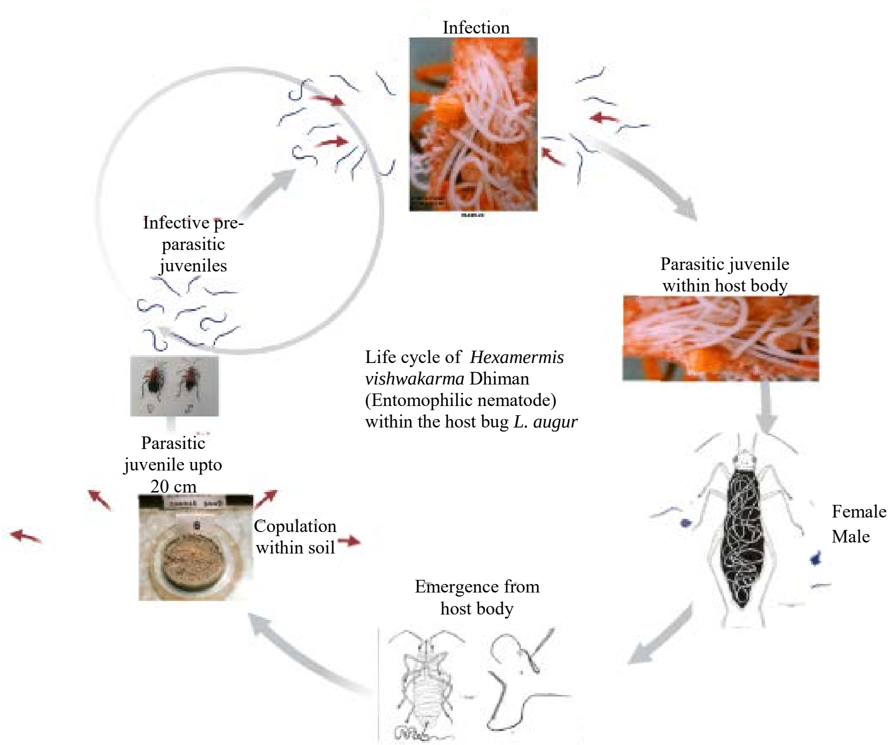

Fig. 2: Life cycle of entomophilic nematodes

Orthopteran mermithid: The earliest and best-known insect parasite of insects is *Mermis nigrescens* from grasshoppers. It was discovered more than 350 years ago and *M. subnigrescens* was described subsequently by Cobb[10] in the United States. Both species have been a matter of controversy regarding synonymy. Nickle[11] clarified afroj and forms of the synonymy. The *Mermis nigrescens* is a common parasite of locusts and grasshoppers. The 48 species of grasshoppers and locusts are hosts of nematode. These insects become infected with *M. nigrescens* when sitting on vegetation having embryonated nematode eggs. The larva hatches in the midgut within an hour of ingestion and then penetrates the gut wall to enter the haemocoel. Here it feeds by osmosis and grows in size and after attaining full size, protrudes out by making a hole in the exoskeleton.

Indian scenario

Orthopteran mermithid: Kooyman[12], reported for Biological Control of the Red Locust, by Nomadacris septemfasciata (Serville) (Orthoptera). Stubbins *et al.*[13] recorded *Agamermis* infection in South Carolina.

However, the incidence of parasitism varies from place to place and year to year. Bhatnagar *et al.*[7] and Choo *et al.*[14] studies on *Agamermis unka* (Nematoda: Mermithidae), a parasite of the brown planthopper *Nilaparvata lugens*.

Hemipteran mermithids: Among the Entomophilic Nematodes, mermithid nematodes remain the least studied on insect hosts. In India, recorded *Hexamermis* spp. parasitizing *Serinetha* (Leptocoris) augur Fabricius (Heteroptera: Coreidae), with a parasitism rate ranging from 30-45% (Fig. 2). Subsequently, Kumkum[15,16] reported a mortality rate of 10-84% in bug populations due to infection by the same nematode species. These findings suggest that *Hexamermis vishwakarma* Dhiman significantly reduces bug populations and holds promise as a potential biocontrol agent.

Furthermore, Poinar[9] recorded *Hexamermis* sp. from Damoh, Madhya Pradesh, parasitizing the pentatomidae bug *Platypus* sp. Additionally, Bhatnagar *et al.*[7] reported *Pentatomimermis* sp. infecting *Nezara viridula*. In India, Kumkum[16] also emphasized the need for successful field trials and suggested deploying natural field-drying methods to effectively control *Leptocoris augur*.

Dipteran mermithid: From India, Rao *et al.*[18] recorded rare parasitism of the bumble bee pollinator, *Bombus impatiens*, by a mermithid nematode, *Pheromermis* sp., *Aelia rostrata* with the entomopathogenic nematode, Poinar[1,21] recorded fossil record of parasitism of *Hexamermis sp.,* Fujimori *et al.*[29] recorded hyperparasitism in mermithid nematodes, Researchers[32,33] recorded mermithid from various larvae of mosquitoes.

There have been reports of *Romanomermis iyengari* parasitizing Anopheles larvae from Bangalore, India. The earliest known accounts of Auchenorrhyncha parasitism by mermithid nematodes in Europe. According to Researchers[34,35] the first instance of adult stable flies being parasitized by *Mermithidae* in Japan.

Coleopteran mermithid: Among the Coleoptera, most of the studies have been carried out on the parasitization of bark beetles and only a few reports are available on mermithids of Coleoptera. Mazza *et al.*[36] reported that in Italy, the Japanese beetle, *Popillia japonica* Newman, is parasitized by *Hexamermis popilliae* sp.

World scenario

Orthopteran mermithids: Rusconi *et al.*[32] recorded nematodes parasitizing grasshoppers in the Pampean Region, Argentina. Hayes and De Coursey[37] recorded 13.9% parasitism in adults and 22.6% in nymphs of *M. differentials* and *M. femurrubrum* by *M. nigrescens* and *A. decaudata* in Illinois and Indiana. *M. nigrescens* is probably the more important of the two mermithid parasites in the U.S., which can withstand a wider range of soil and climatic conditions. In Southeastern Australia, Baker and Poinar[12] found the parasitoid *Agamermis catadecaudata* of the Orthoptera. According to Researchers[13,37], studied *Agamermis* infection in South Carolina. Reserchers[38,39] reported mermithid as control agents of grasshoppers and locusts. Gordon and Webster[40] reported physiological host parasitic relationship of *Mermis nigrescens* with its host, *Schistocerca gregaria* (Table1).

Rusconi *et al.*[41] recorded nematodes parasitizing grasshoppers in the Pampean Region of Argentina.

Choo *et al.*[14] studied parasitism of brown and whitebacked planthoppers by the mermithid nematode *Agamermis* in rice in Korea.

Hemipteran mermithids: The mermithid nematodes are the least known from this group of insects. Yoshiga, *et al.*[39] reported nematodes exploiting *P. japonensis*. In Filippi, Jouvenaz and Wojcik[42] reported mermithid in *Solenopsis richteri*, in Argentina. Poinar[43] reported a parasite of a fossil spider*, Heydenius araneus* and Poinar[44] parasitising an aphid in Myanmar amber. Johnson and Kleve[22] also reported *Pentatomermis coptosoma* from a pentatomidae bug, *Coptosoma mucronatus* from South Slovakia. Mermithidae, both terrestrial and semiterrestrial, were also reported by Kaiser *et al.*[45]. Tong *et al.*[46] reported the first record of a mermithid nematode parasitizing winged females of gall forming aphids. Tarla *et. al.*[31] reported the first *Hexamermis* sp. parasitizing *Eurygaster maura* in an overwintering area and also reported *Hexamermis eurygasteri* sp. parasitizing the Sunn Pest, *Eurygaster integriceps* puton in Turkey. Stoffolano[47] reviewed on host specificity of entomophilic nematodes.

However, no efforts have been made to control the bug by mermithids.

Table 1: Worldwide mermithid host records for hemiptera

S. No.	Host	Species	Genus	Locality	References
1	*Acrosternum* *Euschistus*	*hilare* *servus*	*Hexamermis*	United States	Kamminga *et al.*[17]
2	*Aelia rostrata*	Bohemian	*Hexamermis* *Mermis*	Turkey	Tarla *et al.*[19] Johnson[20]
3	*Chinavia*	*hilaris*	*Hexamermis* *Agamermis*	United States	Stubbins *et al.*[13] Kamminga *et al.*[17]
4	*Ablabesmyia*	*monilis*	*Fibromermis roarki*	Northern Minnesota	Johnson and Kleve[22]
5	*Sogatella*	*furcifera*	*Agamermis* *Hexamermis*	Asia	Chao and Kaya[23]
6	*Halyomorpha*	*halys*	*Hexamermis*	Japan	Watanabe *et al.*[24]
7	*Piezodorus*	*guildinii*	Undetermined *Hexamermis* or *mermis*	United States Uruguay United States	Kamminga *et al.*[17] Riberiro and Castiglioni[25] Kooyman *et al.*[12]
8	*Euschistus*	*servus*	Undetermined *Agamermis*	United States	Esquivel[26] Stubbins *et al.*[13]
9	*Platypus* sp.		*Hexamermis*	India	Poinar and Buckely[9]
10	*Euschistus* spp.	-	*Agamermis*	United States	Stubbins *et al.*[13]
11	*Nezara*	*viridula*	Undetermined *Pentatonix*	United States India	Fuxa *et al.*[27]
12	*Megacopta*	*cribraria*	*Agamermis*	United States	Stubbins *et al.*[13]
13	*Halys*	*dentatus*	*Hexamermis*	India	Yadav and Dhiman[28]
14	*Leptocoris*	*augur*	*Hexamermis*	India	Kumkum[15]
15	*Chironomid*	*larvae*	*Hydromermis biesboer*	Lake Ozawindib in Northern Minnesota	Johnson and Kleve[20]
16	*Eurygaster*	*maura*	*Hexamermis*	Turkey	Tarla *et al.*[19]
17	*Eurygaster*	*integriceps*	*Mermis* *Hexamermis*	Turkey Turkey	Dikyar[30] Tarla *et al.*[31]

Hymenopteran mermithids: Poinar *et al.*[48] also made studies on the mermithids of ants. Blackith and Stevenson[49] found 65 parasitized queens out of 710 (9% parasitization in a nest of *V. vulgaris*). In a nest of *V. germanica,* these authors found 35% of a sample of nearly 200 queens to be parasitised, while only 0.25% of 400 males and 4.5% of 67 workers were parasitized from the same nest. However, mermithid parasitism has no marked effect on the wasp population.

Dipteran mermithids: A good work has been carried out on the chironomid infestation by mermithids. Poinar[50] listed 62 chironomid hosts of nematodes, 58 of which were parasitized by mermithids, mainly confined to intersexual development of the adult host fly. Poinar *et al.*[51] discovered a species of *Cretacimermis* and a mermithid of biting midges, Heleidomermis cataloniensis n. sp., from *Culicoides circumscriptus* Kieffer in Spain. Poulin[52] observed parasites's evolutionary ecology. Keiser *et al.*[45] have studied Urbanization in sub-Saharan Africa and implications for malaria control. Petersen and Willis[53] reported *Diximermis peterseni* works as biocontrol agent of Anopheles mosquito larvae. Bailey *et al.*[54] have prepared mass rearing of the mermithid parasite and experimental release of a mermithid nematode to control showing application of mermithids as control agents of mosquitoes.

Orthopteran mermithids: According to Kumar *et al.*[57] mermithids play an important role in the management of agricultural and household insect pests.

Kaburaki and Imamura[58] observed that 76% population of the rice Borer *Chilo simplex* was affected by mermithids. *H. truncata* and *A. zuimushi* kill more than 90% population of lepidopteran larvae. A complete list of different lepidopteran hosts of *H. truncata* has been presented by Nickle[1].

Lepidopteran mermithids: Various mermithid nematodes of Lepidoptera have been recorded in literature, which include *M. nigrescens*, *M. indica, Hexamermis truncata*, *H. microamphidis, H. ferghanensis, H. cavicola, H. arvalis, Agamermis decaudata, Amphimermis elegans, A. bongongae and A. zuimushi.* Infecting Iranian lepidopteran larvae with *Hexamermis albicans* was initially reported by Nikdel *et al.*[59].

The parasitic larvae of mermithids develop on reserves that normally provide the fat body of the host, retard development and delayed pupation of the host and each host is eventually killed when the fully grown parasitic stage departs. In the field population incidence of parasitism is very high.

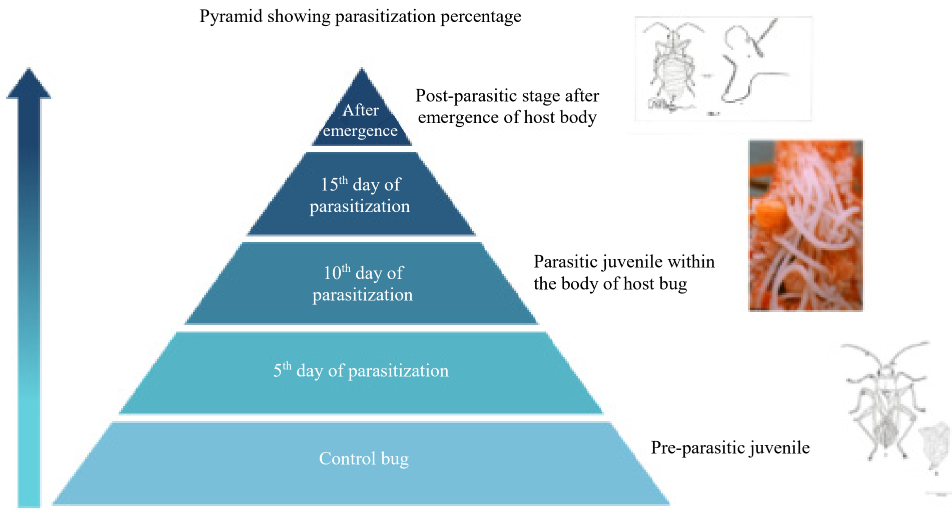

Fig. 3: Parasitisation percentage by *Hexamermis vishwakarma* Dhiman

Hymenopteran mermithid: Ant's species are reported as hosts of mermithids. Wheeler[60] was the first to describe morphological and behavioural modifications induced by mermithid parasitism of an ant *P. commutata*. Up till now, 19 genera of ants are known as a host of mermithids.

Dipteran mermithid: Mosquitoes, black flies, chironomids and a few other dipterans are reported as hosts of mermithids. Various species of mermithid nematodes have proved to show great potential for biological control of different species of mosquitoes and black flies.

Among other species of mosquitoes, mermithids, *Romanomermis culicivorax* has been extensively studied and field trials were made to control the mosquitoes by using this species. mosquitoes larvae are killed before pupation when the parasitic juvenile emerges. This species of nematode infests near about 16 species of mosquitoes belonging to six genera. In 1971, *R. culicivorax* was released at 10 sites and 33% parasites were recorded in the second, third and fourth post-instar larvae[54]. Gordon *et al.*[61] explain mermithid nematodes for the biocontrol of blackflies.

In 1973, more extensive field trials were made with 76% parasitism. Mosquitoes after field applications, 85% of parasites were recorded by Petersen[55,56], Levy and Miller[56] observed 96% parasitism of 4 flood water mosquito species with a rate of 3600 per square metre of surface area. Nickle[11] recorded 50-100% parasitism in the larvae in Maryland. Many other studies have revealed that proper use of mermithid can be effective in controlling mosquito populations. Moreover, mermithid nematodes are the most promising true biocontrol agents for mosquitoes at present.

The population of black flies is also checked to a great population of black flies is also check to a great extent by mermithids. According to Poinar[62], the history of mermithids is found in 67 species of Simulids. Incidence 50-99% of mermithid parasites in simulated populations in the United States and USSR[1]: Helden[34] records of mermithid nematode parasitism of Auchenorrhyncha in Europe. Poinar[63] recorded a parasite *Epidermis cozii* of *Anopheles funestus* in West Africa. Shapiro-Ilan *et al.*[64] reported efficacy of *Steinernema carpocapsae* for control of *Synanthedon pictipes.*

Coleopteran mermithids: Among the Coleoptera, most of the studies have been carried out on the parasitisation of bark beetles and only a few reports are available on mermithids of Coleoptera. Glaser and Henry[65] also observed a mermithid from a Japanese beetle larva, *Popillia japonica*.

Košulic and Mašová[66] also recorded the First Report on Mermithid Parasitism in a Southeast Asian Spider. New reports of spider parasitoids belonging to the family Mermithidae in Brazil and Peru. Poinar and Benton[67] reported *Aranimermis aptispicula* a parasite of spiders.

FUTURE RECOMMENDATIONS

Experimental and field trial to control *Leptocoris augur* by using *Hexamermis vishwakarma* Dhiman has yielded significant success[68,69] at Saharanpur UP (India) there is a dire need to try mermithids for the control of mosquitoes, agricultural and forest paste and other nuisance insects in India and to achieve this entomologist have to play a vital role (Fig. 3).

CONCLUSION

Entomophilic Nematodes, particularly entomopathogenic nematodes (EPNs), show great promise as eco-friendly biocontrol agents against a wide range of insect pests. Despite their global application, their potential in India, especially that of mermithids, remains underutilized. These nematodes are unique in their ability to invade the insect body cavity, leading to rapid insect mortality. Mass production through *in vivo* and *in vitro* methods enables their use in small-scale fields and controlled environments. Further research on their ecological interactions and application strategies is essential to optimize their biocontrol efficiency.

SIGNIFICANCE STATEMENT

This study identified that *in vivo* and *in vitro* mass multiplication of Entomophilic Nematodes (EPNs), could be beneficial for the control of dangerous insect pests. This study will assist researchers in uncovering critical areas of this feature to use EPNs as the ideal organisms for managing insects in different agricultural systems, of the biocontrol potential of Entomophilic Nematodes that have remained unexplored by many. Consequently, a new theory on biocontrol potential by mass culture of Entomophilic Nematodes may be developed.

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