

Management of Dipteran pests through Entomopathogenic Nematodes

Abstract:

Dipteran insects are most destructive agricultural pests, They are vectors of many diseases. EPNs have been reported as a potential biocontrol agent against many Dipteran insect pests. However, several biotic and abiotic factors along with method of application influence the bioefficacy of this organism against Dipteran insects.

Key words:Entomopathogenic nematodes (EPNs), Dipteran insect, biocontrol agent, Application method

Introduction

The order Diptera is a successful group of insects with greatly diversified number of insects with more than 125,000 species. They are coevolved in association with plants and animals. They are small to medium sized insects with soft bodies with a single pair of wing, commonly known as flies. Besides being agricultural pests, they are vectors of many diseases. Some of them are fruit flies (Tephritidae and Drosophilidae), leaf miners (Agromyzidae) or gall former (Cecidomyiidae). Larvae of some Dipteran pests are predatory in nature. Some are obligate parasites of mammals and livestock. Much effort has been made to control this pest through chemical treatment. The application of pathogenic organisms has been advocated as an ecofriendly control method for insect pests. Entomopathogenic nematodes (EPNs) offer an ecofriendly and IPM compatible alternative to chemical insecticides for the control of Dipteran insects.

Habitat of Dipteran insect

Maggots or grubs are the larval stage of the order Diptera are the most important agriculturally ; they are found in many habitats viz., in any kind of water, soil, in plant tissue or beneath bark or stones, in organic matter. Adults also feed on plant or animal juices or other insects.

Management

Insecticides are commonly used to control of feeding and disease transmission. However, some new technologies like using of novel insecticides, repellants, sterile insect technology have been advocated for the management of Dipteran pests. However, growing resistance against some

insecticides along with environmental pollution and the lack of effective biological control alternatives make using Entomopathogenic nematodes (EPNs) against many insect pests of agricultural crops.

Entomopathogenic nematodes(EPNs)

Entomopathogenic nematodes (EPNs) are the obligate parasites of insect pests. The 3rd stage juveniles are the infective stages that are free-living, non-feeding under the families of Steinernematidae and Heterorhabditidae). Members of both families are associated with mutualistic bacteria of the genera *Xenorhabdus* (for Steinernematidae) and *Photorhabdus* (for Heterorhabditidae). IJs locate the host by detecting the insect excretory products, carbon dioxide levels, temperature gradients and movement of the host. IJs penetrate the host through natural openings, mouth, anus or spiracles, or through the cuticle. Once they enter the hemocoel, they release the bacteria which multiply and kill the host within 24-48 hrs. In field application, a concentration of $2.5-5 \times 10^9$ IJs/ha are recommended to give effective control. ENPs have a great potential to be used in integrated pest management programs. However, the susceptibility of insect pests varies depending on the selectivity and applied rates of EPN species. Temperature, moisture, aeration and soil type, the species of EPN, stage of target insects and soil fauna are important factors affecting the bioefficacy of EPNs (Koppenhofer. et al., 2020).

Control potentialities of EPNs(Table.1)

Fungus gnat larvae damage cuttings of various ornamentals and reduce root weight and vigor of a wide range of ornamentals. Larval feeding is believed to predispose the plants to attack by pathogenic fungi. Determining an appropriate concentration, application timing and temperature is crucial in the cost effective control of fungus gnats in greenhouse production. A single application of *S. feltiae* (2.5×10^6 IJs/m²) against the second, third, and fourth instar larvae and at temperatures below 25°C produced consistently high level of control. The potting media affect the survival and infectivity of the nematodes.

Leafminers, *Liriomyza* spp. are among the major pests of field and glasshouse-grown vegetables and ornamental crops worldwide. Larval mining and adult stippling caused by the leafminers destroy leaf mesophyll, decrease the level of photosynthesis, and allow entry of plant pathogens. The use of various species and strains of steinernematids and heterorhabditids against soil-inhabiting prepupae and pupae stages of leafminers produced variable and inconsistent results (Head and Walters, 2003). In general, to achieve reliable control, optimum spray volume is

essential to allow the nematodes to come in contact with the larval stages. Maintaining high relative humidity (above 90%) in the greenhouse and/or moisture on the plants for at least 6–8 h after nematode applications is critical for successful control. The best control of *Liriomyza trifolii* was achieved with 2–4 weekly applications of *S. carpocapsae* or *S. feltiae* at 1×10^6 IJs/m² against the second and the third instar larvae.

Sciarid flies that are found associated with mushrooms throughout the world, *Lycoriella auripila* (Fitch), *Lycoriella mali* (Fitch) and *Lycoriella solani* Winnertz, are the most significant species adversely affecting mushroom cultivation. Larvae feed on the compost and destroy its structure and water retention capacity, which in turn inhibits mycelial colonization causing a significant reduction in mushroom yield. The use of appropriate nematode rates, application site, and application timing for the cost effective management of mushroom flies is economically important for mushroom growers. Several concentrations ranging from 1.5–3.0 $\times 10^6$ *S. feltiae*/m² have been tested to achieve satisfactory control of sciarid infestations.

The phorid fly, *Megaselia halterata* (wood) causes considerable problems for mushrooms. Adults are very strongly attracted to compost or casing in which spawn is running. Larvae feed on mycelia, generally in lower layers of compost. Adult flies become most problematic when they enter the crop soon after spawn run. *S. feltiae* can infect phorid fly larvae in mushroom production houses.

The cabbage maggot, *Delia radicum* (L.), is a cosmopolitan pest of radish, rutabaga and other cole crops. The larvae hatch and tunnel into root tissue and can reduce yield through plant stunting or death. *S. carpocapsae* and *S. feltiae* have been the most commonly used species in field evaluations. Timing and conditions for nematode applications have to be optimal because *D. radicum* larvae are only in the soil for a brief period.

The house fly, *Musca domestica* (L.), is common in animal-rearing farms. Larvae develop in manure and other organic matter and the life cycle can be completed in as little as 7–10 days depending on temperature. In laboratory assays, nematodes that were applied to moist filter paper or animal manure were effective in killing this insect with the most susceptible stages being second and third instar larvae and adults. Baits treated with either *S. feltiae* or *H. megidis* provided significantly greater control of the adult population compared to synthetic chemicals.

Among the fruit fly species, the oriental fruit fly, *Bactrocera dorsalis* (Hendel) is an important insect pest of citrus fruits. Both third instar larvae and pupae are biological stages that persist in

the soil until adult emergence. Several studies have been carried out under laboratory and field conditions showing how ENs can be applied within an area-wide integrated pest management approach to control fruit fly species in orchards and backyard fruit trees.

The infective stages of nematodes, *S. carpocapsae* are sprayed on *Liriomyza*-infested plants. The IJs enter the leafmine via the punctures created by female flies during egg laying or host feeding. Upon contact with the host larva, nematodes are likely to infect the insect via the anus rather than the mouth, but not through spiracles. Evidence from various studies suggests a requisite ambient relative humidity of greater than 90% for nematodes. However, efficacy varies depending on pest species, development stage of the pest, concentrations of nematodes and environmental conditions (Abbas, 2022). Environmental conditions are critical to the survival and virulence of nematodes. entomopathogenic nematodes should be formulated and applied to optimize their performance. The choice of application method may influence how nematodes should be formulated for best results. Infective stage juveniles (IJ) may be applied to foliage using common agrochemical equipment, including hand-held pressurized sprayers, mist blowers, electrostatic or spinning disc systems and aircraft mounted atomizer sprayers.

Table.1. Parasitism of entomopathogenic nematodes against dipteran insects.

Entomopathogenic nematodes	Insects	Infectivity(%)	references
<i>Heterorhabditis bacteriophora</i> , <i>H. indica</i> , <i>Steinernema asiticum</i> , <i>S. carpocapsae</i> , <i>S. glaseri</i> <i>S. feltiae</i>	Oriental fruit fly larvae (<i>Bactrocera dorsalis</i>)	94.97 %	Aatif et al.,2019;2023
<i>S. carpocapsae</i> <i>H. bacteriophora</i> <i>S. feltiae</i> (SN), <i>S. carpocapsae</i> (All), <i>S. riobrave</i> , <i>S. glaseri</i> (NC), <i>H. bacteriophora</i> <i>H. marelatus</i>	Olive fruitfly pupae (<i>Bactrocera oleae</i>)	80%	Torrini et al.,2017
<i>H. bacteriophora</i> , <i>H. indica</i> , <i>H. marelatus</i> , <i>H. zealandica</i> , <i>S. feltiae</i>	The olive fruit fly, third-instar larvae (<i>Bactrocera oleae</i>)	19.1-67.9%	Sirjaniet al.,2009
<i>S. feltiae</i> , <i>S. carpocapsae</i> <i>H. bacteriophora</i>	late third larval instar and pupal stages of <i>Queensland fruit fly</i> (<i>Bactrocera tryoni</i>)	caused significant larval mortality	Aryal et al.,2022
	Third larval instar of <i>Queensland fruit fly</i>	caused significant	Langford et al.,2013

<i>S. feltiae</i>	(<i>Bactroceratryoni</i>) peach fruit fly, (<i>Bactrocerazonata</i>)	larval mortality 100%	Mahmoud. 2007
<i>H.bacteriophora</i> , <i>H. megidis</i> , <i>H. georgiana</i> , <i>H. floridensis</i> , <i>H.indica</i> , <i>S.carpocapsae</i> , <i>S. riobrave</i> , <i>S. feltiae</i> , <i>S. rarum</i> , <i>S. glaseri</i>	larvae, pupae and adultsof <i>Bactrocerazonata</i> B. <i>dorsalis</i>	58.50- 93.06%	Usman et al.,2021; Wakil et al.,2022
<i>H.bacteriophora</i> , <i>H.indica</i> , <i>S.carpocapsae</i> , <i>S.glaseri</i> .	3rd larval instar of house fly (<i>Musca domestica</i>)	43.3-10%	Bream <i>et</i> <i>al.</i> ,2018
<i>S.feltiae</i> SN, <i>S. feltiae</i> UK <i>S. carpocapsae</i> (All) <i>H.bacteriophora</i> (NC) <i>H.megidis</i>	<i>Lycoriellaauripila</i> , <i>L. mali</i> , <i>L. solani</i> <i>Megaseliahalterata</i> Fungus gnats (<i>Bradysiacoprophila</i> , <i>B. difformis</i>)		Grewal & Richardson, 1993; Harris et al., 1995;Scheepma keret <i>al.</i> , 1998; Jagdale <i>et al.</i> , 2004,2007; Jess <i>et al.</i> 2005; Tomalak et al., 2005; Grewal,2007 Grewal & Georgis, 1998; Grewal,2007 Lamba et al. (2008)
<i>Steinernema</i> sp. <i>S. abbasi</i> <i>S. pakistanense</i> <i>H. indica</i>	<i>Megaseliasandhui</i>	13–33%	
<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>H. indica</i> , <i>S.longicaudum</i>	chive maggot (<i>Bradysiaodoriphaga</i>)		Bai et al., 2016; Wu et al.,2017
<i>S. yitgalemense</i> <i>S. feltiae</i> <i>S. jeffreyense</i> <i>S. khoisanae</i>	<i>Bradysia impatiens</i>	52-87%	Katumanyane (2017)

<i>Steinernema sp.</i>				
<i>H. indica</i>				
<i>H. zealandica</i>				
<i>H. bacteriophora</i>				
<i>H. noenieputensis</i>				
<i>S. carpocapsae</i> All,	<i>Liriomyzatrifolii</i>	24-69%		Hara et al.,
<i>S. feltiae</i> MG-14	<i>L. huidobrensis</i>	82%		1993;
<i>Heterorhabditis</i> sp.UK 211	<i>L. bryoniue</i>			LeBeck et al.,
				1993;
				Sher et al.,
				2000;
				Head & Walters,
				2003;
				Tomalak et
				al.,2005;
				Williams &
				Walters,2000
				Williams
				&Macdonald ,
				1995
				Yee and Lacey,
<i>S. riobrave,</i>	fruit flies			2003;
<i>S. carpocapsae,</i>	(<i>Rhagoletisindifferens,</i>			Toledo et al.,
<i>S. feltiae</i>	<i>Anastrephaludens</i>)			2005
<i>S.carpocapsae</i> ALL <i>S. feltiae</i> SN	Pupal stage of the apple	42.9-		Usman et
,	maggot	73.8%		al.,2020
<i>S. riobrave</i> 355 ,	(<i>Rhagoletispomonella</i>)			
<i>S. glaseri</i> VS ,				
<i>H.bacteriophora</i> VS				
<i>H.indica</i> HOM1				
<i>H.megidis</i> UK211				
<i>H. bacteriophora,</i>	European cherry fruit	40-95%		Kepenekci et
<i>H. marelatus,</i>	fly			al., 2015
<i>S.carpocapsae,</i>	(<i>Rhagoletiscerasi</i>)			
<i>S. feltiae</i>				
<i>S.bicornutum,</i>	European cherry fruit	54%		Koppler et
<i>S. carpocapsae,</i>	Fly (<i>Rhagoletiscerasi</i>)			al.,2003
<i>S. carpocapsae</i> China, <i>S.</i>				
<i>feltiae</i> <i>H.bacteriophora</i>				
<i>H. bacteriophora</i> UWS1	Crane fly	28-65%		Ansari &
<i>H.bacteriophora,</i>	(<i>Tipulapaludosa</i>)			Butt,2012;
<i>S. carpocapsae ,</i>				Oestergaardet
<i>S.feltiae</i>				al., 2006;
				Simard <i>et al.</i> ,
				2006
<i>S.feltiae</i>	Cranefly larvae	13-90%		Ehlers

<i>S. anomali</i>	(<i>Tipulapaludosa</i>)		&Gerwien, 1993
<i>S. affinis</i>			
<i>S. feltiae</i>	Cranefly larvae (<i>Tipulapaludosa</i> , <i>Tipula oleracea</i>)	L1 susceptible stage	Peters &Ehlers,1994
<i>S. riobrave</i>	Mediterranean fruit fly or	50-100%	Gazit <i>et al.</i> , 2000;
<i>S. carpocapsae</i>	medfly		Lindegren <i>et al.</i> , 1990
<i>S. feltiae</i>	(<i>Ceratitis capitata</i>)		Chergui <i>et al.</i> 2019.
<i>S. feltiae</i> -SF-MOR9, <i>S. feltiae</i> -SF-MOR10	Medfly (<i>Ceratitis capitata</i>)	80%	Mokrini <i>et al.</i> ,2020
<i>H. bacteriophora</i> -HB-MOR7			
<i>Heterorhabditis</i> sp. RSC01 ,	Third-instars of	26.7-	Rohde <i>et al.</i> ,2010
<i>S. carpocapsae</i> ALL	Mediterranean fruit fly, (<i>Ceratitis capitata</i>)	96.7%	
<i>S. carpocapsae</i> ALL	larvae and pupae of	50-80%	Rohde <i>et al.</i> ,2020;
<i>H. amazonensis</i> JPM4	Mediterranean fruit fly (<i>Ceratitis capitata</i>)		
<i>H. bacteriophora</i> HB, <i>H. amazonensis</i> IBCB-n24, <i>S. carpocapsae</i> IBCB-n02, <i>S. rarum</i> PAM-25, <i>S. glaseri</i> IBCB-n47, <i>S. brazilense</i> IBCB-n06	pupae of Mediterranean fruit fly (<i>Ceratitis capitata</i>)	60-80%	Jean-Baptiste <i>et al.</i> ,2021
<i>H. noenieputensis</i> , <i>H. indica</i>	Mediterranean fruit fly or Medfly,		James <i>et al.</i> ,2018
<i>H. bacteriophora</i> <i>S. yirgalemense</i>	(<i>Ceratitis capitata</i> , <i>Ceratitis rosa</i>)		
<i>H. bacteriophora</i> HP88 <i>S. carpocapsae</i> All	Full-grown larvae and pupae of Mediterranean fruit fly (<i>Ceratitis capitata</i>) Peach fruit fly, (<i>Bactrocera zonata</i>)	44.8-76.8%	Nouh &Hussein ,2014
<i>H. bacteriophora</i> AS1, <i>H. bacteriophora</i> HP88, <i>S. carpocapsae</i> ALL, <i>S. riobrave</i> ML29	Late third instars of the medfly, (<i>Ceratitis capitata</i>)	caused significant mortality	Shaurubet <i>al.</i> ,2015
<i>H. bacteriophora</i> , <i>H. zealandica</i> <i>S. khoisanae</i>	Larvae, pupae and adults of (<i>Ceratitis capitata</i> , <i>Ceratitis rosa</i>)	caused significant mortality	Malan &Manrakhan, 2009
<i>S. weiseri</i> , <i>S. feltiae</i> 09-31 <i>S. carpocapsae</i>	Last instar larvae of Mediterranean fruit fly or Medfly,	50-97%	Karagoz <i>et al.</i> ,2009

<i>H. bacteriophora</i> <i>S. feltiae</i> <i>S. carpocapsae</i> <i>S. riobravivis</i> <i>H. bacteriophora</i> <i>H. marelatus</i>	(<i>Ceratitis capitata</i>) Third instars of Western cherry fruit fly (<i>Rhagoletisindifferens</i>)	caused significant mortality	Patterson & Lacey, 1999
<i>Heterorhabditis</i> sp. LPP17, <i>Heterorhabditis</i> sp. LPP14 <i>H. baujardi</i> LPP7 <i>S. feltiae</i>	Third instar larvae of (<i>Ceratitiscapitata</i>)	90- 98.5%	Minas et al.,2016
<i>H. bacteriophora</i> RS88 <i>S. riobravae</i> RS59	South American fruit fly, (<i>Anastrephafraterculus</i>)	20-51.3%	Barbosa- Negrisoli et al.,2009
<i>H.bacteriophora</i> <i>H.indica</i> <i>S.feltiae</i> <i>H. taysearae</i> Azohoue2 (= <i>H. sonorensis</i>) <i>S.kandii</i> <i>H.bacteriophora</i>	Caribfly (<i>Anastrepha suspense</i>) Larvae and pupae of mango fruit fly (<i>Bactrocera</i> <i>dorsalis</i>) The cabbage maggot (<i>Delia radicum</i>)		Heve et al.,2018 Godjo et al.,2021. Sharifi-Far et al.,2018 Chen et al.2003
<i>S. carpocapsae</i>	<i>Drosophilamelanogaster</i> larvae	Capable of infecting and killing	Pena et al.,2015
<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. kraussei</i> <i>H. bacteriophora</i> <i>S.carpocapsae</i>	spotted wing drosophila (<i>Drosophila suzukii</i>) cucurbit fly (<i>Dacus ciliates</i>)	emergence of flies was significantl y reduced. 12-28%	Garriga et al.,2018 Hübner et al.,2017 Kamali et al.,2013

Conclusion

Different species of entomopathogenic nematodes have distinct temperature niches for activity and may also respond differently to moisture availability. Therefore, isolation of local species and strains for use in specific environments are important prerequisite as biopesticides. Increased understanding of nematode biology, host range and concurrent advances in commercial production, storage and formulation, have led to nematode-based biopesticides safe alternatives to chemical insecticides. Successful use requires that the ecology of the target is matched to the activity of infective juveniles; in practice, targets are protected from environmental extremes,

applications are timed to coincide with susceptible host stages and favourable weather conditions and nematodes are able to rapidly locate and infect hosts. In addition to performance, factors including cost, availability, compatibility within integrated strategies and alternative options for organic growers will ultimately determine the extent to which nematodes are used against Dipteran insect pests.

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UNDER PEER REVIEW