Management of Dipteran pests through Entomopathogenic Nematodes

Abstract:

Dipteran insects are most destructive agricultural pests. They are also vectors of many diseases of human and animal. Much effort has been made to control this pest through chemical treatment. The application of biological control agents has been advocated as an ecofriendly control method for insect pests. Entomopathogenic nematodes (EPNs) are obligate insect parasites that can be effective biocontrol agents for many agricultural pests including many Dipteran insect pests. EPNs pose much less threat to the environment than chemical pesticides. However, several biotic and abiotic factors along with method of application influence the bioefficacy of this organism against Dipteran insects. This review paper provides an overview of developments in entomopathogenic nematode research and evaluation of their potential for use against Dipteran insect pests.

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

INTRODUCTION

The order Diptera (True flies) is a successful group of insects with greatly diversified number of insects with more than 153,000 species [1]. They are small to medium sized insects with soft bodies with a single pair of wing, commonly known as flies. They are coevolved in association with plants and animals. The order includes many familiar fly species of highly economic importance as pests of plants. Besides being agricultural pests, they are vectors of many diseases of man and animal; some of the Dipteran insects are pollinators of flowers. At least 71 of 150 Dipteran families include flies that feed on flowers as adults [2]. 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. 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. Dipteran pest control has traditionally relied upon chemical pesticides; however concerns

about public safety, soil and water pollution, insecticide resistance, effects on non-target organisms, and enhanced biodegradation of pesticides, have increased the pressure to shift from chemical management to alternative control strategies like using of novel insecticides, repellants, sterile insect technology, and biological control agents. Among the biological control agents, Entomopathogenic nematodes (EPNs) offer an ecofriendly and IPM compatible alternative to chemical insecticides [3]. Several laboratory and field experiments documented the suitability of EPNs for the management of Dipteran insects.

ENTOMOPATHOGENIC NEMATODES (EPNs)

EPNs of the families Heterorhabditidae and Steinernematidae have only one free living stage, the infective juvenile stage (IJ) [4] .Members of both families are associated with mutualistic bacteria of the genera *Xenorhabdus* (for *Steinernema* : Steinernematidae) and *Photorhabdus* (for *Heterorhabditis* : Heterorhabditidae) [5,6]. IJs locate the host by detecting the insect excretory products, carbon dioxide levels, temperature gradients and movement of the host. The IJs seek out and enter insect hosts via the spiracles, mouth, anus, or intersegmental membranes. Once they enter the hemocoel, they release the bacteria which multiply and kill the host within 24-48 hrs. They have a broad host range and are safe to the environment. Entomopathogenic nematodes can be easily mass-produced and applied using conventional spray equipment. 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 (IPM) programs. However, efficacy varies depending on the species of EPN, concentrations of nematodes ,target pest species, development stage of the pest, and environmental conditions like temperature, moisture, aeration, soil type, and soil fauna[7,8].

EFFICACY OF EPNS AGAINST DIPTERAN PESTS (Table.1)

Various laboratory and field experiments documented different degrees of susceptibility of Dipteran pest species to EPNs. The application of EPNs in the soil beneath the tree canopy can kill a significant number of the soil dwelling stages of Dipteran insects. It has been observed that most of the studies involved simple laboratory bioassays with native strains of EPNs. Commercial formulation of the native strains and their availability to farmers are the sustainable solution for the biocontrol of Dipteran pests. The feasibility of this approach can be further improved if adjuvants, such as wetting agents that enhance nematode efficacy. By trapping their application targets (hot spots) within the orchard is a cost effective approach. Determining an

appropriate concentration of IJs of EPN, application timing (off-season or early season), single application or multiple application, and temperature is crucial in the cost effective control of Dipteran pests. In greenhouse production, a single application of *S. feltiae* (2.5×10^6 IJs/m^2) at temperatures below 25^oC against the second, third, and fourth instar larvae of fungus gnats consistently showed high level of control[9]. The use of various species and strains of EPNs against soil-inhabiting prepupae and pupae stages of leafminers produced variable results. Maintaining high relative humidity (above 90%) with optimum spray volume in the greenhouse or on the plants for at least 6–8 h after nematode applications is essential 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/m^2 against the second and the third instars larvae. 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 [10]. *S. feltiae* can infect *Lycoriella auripila*, *Lycoriella mali* and *Lycoriella solani* larvae in mushroom production houses. Several concentrations ranging from 1.5- 3.0×10^6 / m^2 IJs of *S. feltiae* have been tested to achieve satisfactory control of sciarid infestations [11]. *S. carpocapsae* and *S. feltiae* have been the most commonly used species in field evaluations of cabbage maggot, *Delia radicum* .Timing and conditions for nematode applications have to optimal because *D. radicum* larvae are only in the soil for a brief period [12]. In laboratory assays, *S. feltiae* or *H. megidis* were applied to moist filter paper or animal manure against house fly, *Musca domestica*. It was observed that second and third instars and adults were most susceptible stages [13]. Both third instar larvae and pupae are biological stages of the oriental fruit fly, *Bactrocera dorsalis* that persist in the soil until adult emergence. Several studies have been carried out under laboratory and field conditions showing how EPNs can be applied within an area-wide integrated pest management approach to control fruit fly species in orchards and backyard fruit trees[14,15].

Environmental conditions are critical to the survival and virulence of nematodes. The choice of application method may influence how nematodes should be formulated for best results [7]. 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**
Heterorhabditis bacteriophora, *H. indica*, *Steinernema asiticum*, *S. corpocapsae* , *S. glaseri*	Oriental fruit fly larvae (*Bacterocera dorsalis*)	94.97 %	[14,15]
S. feltiae *S. carpocapsae* *H.bacteriophora*	Olive fruitfly pupae (*Bactrocera oleae*)	80%	[16]
S. feltiae (SN), *S. carpocapsae* (All), *S. riobrave*, *S. glaseri* (NC), *H.bacteriophora* *H. marelatus*	The olive fruit fly, third-instar larvae (*Bactrocera oleae*)	19.1-67.9%	[17,77]
H.bacteriophora, *H. indica*, *H.marelatus*, *H.zealandica* , *S. feltiae*	late third larval instar and pupal stages of *Queensland fruit fly (Bactrocera tryoni)*	caused significant larval mortality	[18]
S. feltiae, *S.carpocapsae* *H.bacteriophora*	Third larval instar of *Queensland fruit fly (Bactrocera tryoni)*	caused significant larval mortality	[19]
S. feltiae	peach fruit fly, (*Bactrocera zonata*)	100%	[20]
H.bacteriophora, *H. megidis*, *H. georgiana*, *H. floridensis*,	larvae, pupae and adults of *Bactrocera zonata* *B.dorsalis*	58.50-93.06%	[21] [22,76]

H.indica, *S.carpocapsae,* *S. riobrave,* *S. feltiae,* *S. rarum ,* *S. glaseri*			
H.bacteriophora, *H.indica,* *S.carpocapsae,* *S.glaseri*.	3rd larval instar of house fly (*Musca domestica*)	43.3-10%	[13]
S.feltiae SN, *S. feltiae* UK *S. carpocapsae* (All) *H.bacteriophora* (NC) *H.megidis*	*Lycoriella auripila,* *L. mali,* *L. solani* *Megaselia halterata* Fungus gnats (*Bradysia coprophila ,* *B. difformis*)		[11] [23] [24] [25,26] [27]
Steinernema sp. *S. abbasi* *S. pakistanense* *H. indica*	*Megaselia sandhui*	13–33%	[28]
H. bacteriophora, *S. carpocapsae,* *S. feltiae,* *H. indica,* *S.longicaudum*	chive maggot (*Bradysia odoriphaga*)		[29] [30]
S. yitgalemense *S. feltiae* *S. jeffreyense* *S. khoisanae* *Steinernema sp.*	*Bradysia impatiens*	52-87%	[9]

H. indica *H. zealandica* *H. bacteriophora* *H. noenieputensis*			
S. carpocapsae All, *S. feltiae* MG-14 *Heterorhabditis* sp.UK 211	*Liriomyza trifolii* *L.huidobrensis* *L. bryoniue*	24-69% 82%	[31] [32,73] [33] [10] [34] [35]
S. riobrave, *S. carpocapsae,* *S. feltiae*	fruit flies (*Rhagoletis indifferens,* *Anastrepha ludens*)		[36] [37]
S.carpocapsae ALL *S. feltiae* SN , *S. riobrave* 355 , *S. glaseri* VS , *H.bacteriophora* VS *H.indica* HOM1 *H.megidis* UK211	Pupal stage of the apple maggot (*Rhagoletis pomonella*)	42.9-73.8%	[38]
H. bacteriophora, *H. marelatus,* *S.carpocapsae,* *S. feltiae*	European cherry fruit fly (*Rhagoletis cerasi*)	40-95%	[39]
S.bicornutum, *S. carpocapsae,* *S. carpocapsae* China, *S. feltiae* *H.bacteriophora*	European cherry fruit Fly (*Rhagoletis cerasi)*	54%	[40]
H. bacteriophora UWS1 *H.bacteriophora,*	Crane fly (*Tipula paludosa*)	28-65%	[41] [42]

S. carpocapsae , *S.feltiae*			[43]
S.feltiae *S.anomali* *S.affinis*	Cranefly larvae (*Tipula paludosa*)	13-90%	[44]
S.feltiae	Cranefly larvae (*Tipula paludosa*, *Tipula oleracea*)	L1 susceptible stage	[45]
S. riobrave *S. carpocapsae* *S. feltiae*	Mediterranean fruit fly or medfly (*Ceratitis capitata*)	50-100%	[46] [47] [48]
S.feltiae-SF-MOR9, *S. feltiae*-SF-MOR10 *H.bacteriophora*-HB-MOR7	Medfly (*Ceratitis capitata*)	80%	[49]
Heterorhabditis sp. RSC01 , *S.carpocapsae* ALL	Third-instars of Mediterranean fruit fly, (*Ceratitis capitata*)	26.7- 96.7%	[50,74,75]
S.carpocapsae ALL *H.amazonensis* JPM4	larvae and pupae of Mediterranean fruit fly (*Ceratitis capitata*)	50-80%	[51]
H.bacteriophora HB, *H. amazonensis* IBCB-n24, *S.carpocapsae* IBCB-n02, *S. rarum* PAM-25, *S. glaseri* IBCB-n47, *S. brazilense* IBCB-n06	pupae of Mediterranean fruit fly (*Ceratitis capitata*)	60-80%	[52]
H. noenieputensis, H. indica *H. bacteriophora* *S.yirgalemense*	Mediterranean fruit fly or Medfly, (*Ceratitis capitata, Ceratitis rosa)*		[53]

H.bacteriophora HP88 *S.carpocapsae* All	Full-grown larvae and pupae of Mediterranean fruit fly (*Ceratitis capitata*) Peach fruit fly, (*Bactrocera zonata*)	44.8-76.8%	[54]
H.bacteriophora AS1, *H. bacteriophora* HP88, *S.carpocapsae* ALL, *S. riobrave* ML29	Late third instars of the medfly, (*Ceratitis capitata*)	caused significant mortality	[55]
H.bacteriophora, *H.zealandica* *S.khoisanae*	Larvae, pupae and adults of (*Ceratitis capitata* , *Ceratitis rosa*)	caused significant mortality	[56]
S.weiseri, *S. feltiae* 09-31 *S. carpocapsae* *H. bacteriophora*	Last instar larvae of Mediterranean fruit fly or Medfly, (*Ceratitis capitata)*	50-97%	[57]
S. feltiae *S. carpocapsae* *S. riobravis* *H. bacteriophora* *H. marelatus*	Third instars of Western cherry fruit fly (*Rhagoletis indifferens*)	caused significant mortality	[58]
Heterorhabditis sp. LPP17, *Heterorhabditis* sp. LPP14 *H. baujardi* LPP7	Third instar larvae of *(Ceratitis capitata*)	90- 98.5%	[59]
S. feltiae	*Anastrepha fraterculus*	75%	[60]
H. bacteriophora RS88 *S. riobravae* RS59	South American fruit fly, (*Anastrepha fraterculus*)	20-51.3%	[61]
H.bacteriophora *H.indica*	Caribfly (*Anastrepha suspense*)		[62]

S.feltiae			
H. taysearae Azohoue2 *(=H. sonorensis)* *S. kandii*	Larvae and pupae of mango fruit fly (*Bactrocera dorsalis*)		[63].
H.bacteriophora	The cabbage maggot (*Delia radicum*)	Caused higher levels of infection	[12] [64]
S. carpocapsae	*Drosophila melanogaster* larvae	Capable of infecting and killing	[65]
H. bacteriophora, S. carpocapsae, S. feltiae, S. kraussei	spotted wing drosophila (*Drosophila suzukii*)	emergence of flies was significantly reduced.	[66] [67]
H. bacteriophora *S.carpocapsae*	cucurbit fly (*Dacus ciliates*)	12-28%	[68]Kamali et al.,2013

CONCLUSION

Increased understanding of nematode biology, host range and simultaneous advances in commercial production, storage and formulation, have led to nematode-based biopesticides safe alternatives to chemical insecticides[69,70].Different species of EPNs have distinct temperature niches for activity and may also respond differently to moisture or relative humidity availability. Therefore, isolation of local species and strains for use in specific environments are important prerequisite as biopesticides. Successful use requires that the ecology of the target is matched to the activity of infective juveniles; as the target pests 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 [71]. 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 [72,78-80]. To make entomopathogenic nematodes more successful, realistic strategies through genetic engineering [81], IPM programs, and new delivery systems

and/or training programs to overcome their inherent cost, formulation instability, and limited field efficacy toward the Dipteran pests are needed[82,83].

ACKNOWLEDGEMENTS

Deepest gratitude to the Associate Dean, SCS College of Agriculture, AAU, Dhubri, for his encouragement during the preparation of the manuscript.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Thompson FC. Biosystematic Database of World Diptera.2005.Verson 7.5 .http: // www.diptera.org/biosys.htm.
2. Larson BMH, Kevan PG, Inouye DW. Flies and flowers: The taxonomic diversity of anthophiles and pollinators. The Canadian Entomologist 2001; 133(4):439-465.
3. Grewal PS, Koppenhöfer AM, Choo HY. Lawn, turfgrass and pasture applications. In: Grewal PS, Ehler RU, Shapiro-Ilan DI, Editors. Nematodes As Biocontrol Agents. CABI Publishing, Wallingford, 2005.
4. Shapiro-Ilan DI, Hazir S, Glazer I.Basic and applied research: Entomopathogenic nematodes. In: L. A Lacey. Editor. Microbial agents for control of insect pests: from discovery to commercial development and use. Amsterdam: Academic Press, 2017; 91-105. doi:10.1016/b978-0-12-803527-6.00006-8.
5. Tailliez P, Pages S, Ginibre N, Boemare N. New insight into diversity in the genus *Xenorhabdus*, including the description of ten novel species. *Internat. J. System. Evol. Microbiol.* 2006;56: 2805-2818
6. Akhurst RJ, Mourant RG, Baud L, Boemare NE. Phenotypic and DNA relatedness between nematode symbionts and clinical strains of the genus *Photorhabdus* (Enterobacteriaceae). *Int. J. Syst. Bacteriol*. 1996; 46: 1034-1041.
7. Koppenhofer AM, Shapiro-Ilan DI. Hiltpold I. Entomopathogenic nematodes in sustainable food production. *Front. Sustain. Food Syst.* 2020; 4: 125. https://doi.org/10.3389/fsufs.2020.00125
8. Abbas MST. Pathogenicity of entomopathogenic nematodes to dipteran leaf miners, house flies and mushroom flies. *Egypt J. Biol. Pest Control.* 2022: 2:76. https://doi.org/10.1186/s41938-022-00566-y

9. Katumanyane A. Prospects for using EPNs as a bio-control agent against fungus gnats, *Bradysia* spp. in nursery and glasshouse crops. M.Sc. Thesis, Faculty of Agrisciences, Stellenbosch University, South Africa. Stellenbosch University.2017. https:// schol ar. sun. ac. Za.

10. Head J, Walters KFA. Augmentation biological control using the entomopathogenic nematode *Steinernema feltiae* against the South American leafminer *Liriomyza huidobrensis*. Conference: USDA-Forest Service, 1st International Symposium on Biological Control of Arthropods, 2003;136-140.10.13140/2.1.2799.5528. DOI:10.13140/2.1.2799.5528.

11. Grewal PS, Richardson PN. Effects of application rates of *Steinernema feltiae* (Nematoda: Steinernematidae) on control of the mushroom sciarid fly *Lycoriella auripila*. *Biocontrol Sci.Technol.* 1993; 3: 29-40. https:// doi.org /10.1080 /09583159309355256

12. Sharifi-Far S, Shapiro-Ilan DI, Brownbridge M, Hallett RH. The combined approach of strain discovery and the inbred line technique for improving control of *Delia radicum* with *Heterorhabditis bacteriophora*. *Biol.Control.* 2018; 118:37-43. doi: 10.1016/j.biocontrol.2017.12.004

13 .Bream AS, Fouda MA, Shehata IE, Ragab SH. Evaluation of four entomopathogenic nematodes as biological control agents against the housefly, *Musca domestica* L. (Diptera: Muscidae). *Egypt. Acad. J. Biolog. Sci.* (A. Entomology) 2018;11(1): 79-89. 10.21608/EAJB.2018.11982

14. Aatif HM, Hanif MS, Ferhan M, Raheel M, Shakeel Q, Ashraf W et al., Assessment of the entomopathogenic nematodes against maggots and pupae of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae), under laboratory conditions, Egypt. J. Biol. Pest Control. 2019: 29: 51. https: //doi. org/10.1186/s41938-019-0154-4.

15. Aatif HM, Afzal A, Idrees A, Mansha MZ, Shahid Hanif CM, Ali Y, et al.. Entomopathogenic nematodes for the control of oriental fruit fly *Bacterocera dorsalis* (Diptera: Tephritidae). *Journal of King Saud University - Science.* 2023:35:102428. https://doi.org /10.1016/ j.jksus. 2022.102428.

16. Torrini G, Mazza G, Benvenuti C, Roversi PF. Susceptibility of olive fruitfly, *Bactrocera oleae* (Diptera: Tephritidae) pupae to entomopathogenic nematodes. *J. Plant Prot. Res.* 2017; 57: 318-320.DOI: https://doi.org/10.1515/jppr-2017-0030

17. Sirjani FO, Lewis EE, Kaya HK. Evaluation of entomopathogenic nematodes against the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). *Biol.Control* . 2009; 48:274-280. https: //doi. Org /10.1016/j.biocontrol.2008.11.002

18. Aryal S, Nielsen UN, Sumaya NH, Wilson C, Riegler M. Virulence, penetration rate and reproductive potential of entomopathogenic nematodes from eastern Australia in Queensland fruit fly, *Bactrocera tryoni*. *Biol. Control.* 2022;169, 104871. https://doi.org/10.1016/j.biocontrol.2022.104871

19. Langford EA, Nielsen UN, Johnson SN, Riegler M. Susceptibility of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), to entomopathogenic nematodes. *BioControl*.2013; 96: 34-39. DOI:10.1016/j.biocontrol.2013.10.009.

20. Mahmoud MF. Combining the botanical insecticides NSK extract, NeemAzal T 5%, Neemix 4.5% and the entomopathogenic nematode *Steinernema feltiae* Cross N 33 to control the peach fruit fly, *Bactrocera zonata* (Saunders). *Plant Prot. Sci*. 2007; 43(1):19-25.

21. Usman M, Gulzar S, Wakil W, Piñero JC, Leskey TC, Nixon LJ et al., Potential of entomopathogenic nematodes against the pupal stage of the apple maggot *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). *J.Nematol*.2020; 52, e2020-79. DOI: 10.21307/jofnem-2020-079

22. Wakil W, Usman M, Piñero JC, Wu S, Toews MD, Shapiro-Ilan DI.. Combined application of entomopathogenic nematodes and fungi against fruit flies, *Bactrocera zonata* and *B. dorsalis* (Diptera: Tephritidae): Laboratory cups to field study. *Pest Manag. Sci.* 2022; 78: 2779-2791.https://doi.org/10.1002/ps.6899.

23. Harris MA, Oetting RD, Gardner WA. Use of entomopathogenic nematodes and a new monitoring technique for control of fungus gnats, *Bradysia coprophila* (Diptera: Sciaridae), in floriculture. *Biol. Control.* 1995;5(3): 412-418. doi: 10.1006/bcon.1995.1049.

24. Scheepmaker JWA, Geels FP, Rutjens AJ, Smits PH, Van Griensven LJ. Comparison of the efficacy of entomopathogenic nematodes for the biological control of the mushroom pests *Lycoriella auripila* (Sciaridae) and *Megaselia halterata* (Phoridae). *Biocontrol Sci. Technol.* 1998; 8:277-287. https:// doi.org /10. 1080 /09583159830342

25. Jagdale GB, Casey ML, Grewal PS, Lindquist RK. Application rate and timing, potting medium, and host plant effects on the efficacy of *Steinernema feltiae* against the fungus

gnat, *Bradysia coprophila*, in floriculture. *Biol.Control*. 2004; 29:296-305. DOI: 10. 1016 /S1049-9644(03)00164-6

26. Jagdale GB, Casey ML, Canas L, Grewal PS. Effect of entomopathogenic nematode species, split application and potting medium on the control of the fungus gnat, *Bradysia difformis* (Diptera:Sciaridae), in the greenhouse at alternating cold and warm temperatures.*Biol.Control.*2007; 43:23-30. DOI:10.1016/j. biocontrol.2007.03.013
27. Jess S, Schweizer H, Kilpatrick M. Mushroom applications. In: Grewal PS, Ehler RU, Shapiro-Ilan DI, Editors. Nematodes As Biocontrol Agents. CABI Publishing, Wallingford, 2005.
28. Lamba JS, Walia KK, Mrig K, Walia RK. Infectivity and virulence of indigenous entomopathogenic nematodes to mushroom phorid fly, *Megaselia sandhui*. *JBC*.2008; 22(2):411-416
29. Bai GY, Xu H, Fu YQ, Wang XY, Shen GS, Ma HK et al. A comparison of novel entomopathogenic nematode application methods for control of the chive gnat, *Bradysia odoriphaga* (Diptera: Sciaridae). *J. Econ. Entomol.*2016; 109(5): 2006–2013. https://doi.org/10.1093/jee/tow162
30. Wu H, Gong Q, Fan K, Sun R, Xu Y, Zhang K. Synergistic effect of entomopathogenic nematodes and thiamethoxam in controlling *Bradysia odoriphaga* Yang and Zhang (Diptera: Sciaridae). *Biol. Control*. 2017;111:53-60. 10.1016/j.biocontrol.2017.05.006.
31. Hara AH, Kaya HK, Gaugler R, LeBeck LM, Mello CL. Entomopathogenic nematodes for biological control of the leafminer, *Liriomyza trifolii* (Dipt. Agromyzidae) (Rhabditida: Steinernematidae). *Entomaphaga.* 1993; 38:359-369. PMID : 23482993.
32. LeBeck LM, Gaugler R, Kaya HK, Hara AH, Johnson MW. Host stage suitability of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *J.Invertebr.Pathol.* 1993; 62:58-63.
33. Sher RB, Parrella MP, Kaya HK. Biological Control of the Leafminer *Liriomyza trifolii* (Burgess): Implications for intraguild predation between *Diglyphus begini* Ashmead and *Steinernema carpocapsae* (Weiser). *Biol. Control* .2000;17(2):155-163. https://doi.org/10.1006/bcon.1999.0794

34. Williams EC, Walters KFA. Foliar application of the entomopathogenic nematode, *Steinernema feltiae* against leafminers on vegetables. *Biocontrol Sci Technol.* 2000; 10: 61-70. https://doi.org/10.1080/09583150029396.

35. Williams EC, Macdonald OC. Critical factors required by the nematode *Steinernema feltiae* for the control of the leafminers *Liriomyza huidobrensis, Liriomyza bryoniae* and *Chromatomyia syngenesiae*. *Ann.Appl. Biol* .1995; 127: 329-341. DOI:10.1111/j.1744-7348.1995.tb06677.x.

36. Yee WL, Lacey LA. Stage-specific mortality of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to three species of *Steinernema* Nematodes. *Biol. Control.* 2003; 27:349-356. https://doi.org/10.1016/S1049-9644(03)00029-X.

37. Toledo J, Ibarra JE, Liedo P, Gomez A, Rasgado MA, Williams T. Infection of *Anastre phaludens* (Loew) (Diptera: Tephritidae) larvae by *Heterorhabditis bacteriophora* under laboratory and field condition. *Biocontrol Sci.Technol.* 2005; 15:627-634. DOI: 10.1080/09583150500089049.

38. Usman M, Gulzar S, Wakil W,Wu S, Piñero JC, Leskey TC et al. Virulence of entomopathogenic fungi to *Rhagoletis pomonella* (Diptera: Tephritidae) and interactions with entomopathogenic nematodes. *J. Econ. Entomol.* 2020; 113: 2627-2633. https:// doi.org /10.1093/jee/toaa209

39. Kepenekci I, Hazir S, Ozdem A. Evaluation of native entomopathogenic nematodes for the control of the European cherry fruit fly *Rhagoletis cerasi* L.(Diptera: Tephritidae) larvae in soil. *Turk. J. Agric. For.* 2015; 39(1):74-79. . https://doi.org/10.3906/ tar-1403-96.

40. Koppler K, Peters A, Vogt H. Initial results in the application of entomopathogenic nematodes against the European cherry fruit fly *Rhagoletis cerasi* L. (Diptera: Tephritidae). *IOBC / WPRS Bullet* 2003; 23:13-18

41 .Ansari MA, Butt TM. Evaluation of entomopathogenic fungi and a nematode against the soil-dwelling stages of the crane fly *Tipula paludosa*. *Pest Manag. Sci.* 2012. 68: 1337-1344. https:// doi. org/10.1002/ps.3338.

42. Oestergaard J, Belau C, Strauch O, Ester A, van Rozen K, Ehlers RU. Biological control of *Tipula paludosa* (Diptera: Nematocera) using entomopathogenic nematodes (*Steinernema* spp.) and *Bacillus thuringiensis* subsp. *israelensis*. *Biol.Control.* 2006; 39:525-531. DOI:10.1016/j.biocontrol.2006.07.003.

43. Simard L, Belair G, Gosselin ME, Dionne J. Virulence of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against *Tipula paludosa* (Diptera: Tipulidae), a turfgrass pest on golf courses. *Biocontrol Sci. Technol.* 2006;16:789-801. DOI:10.1080/09583150600700073.

44. Ehlers RU, Gerwien A. Selection of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae, Nematoda) for the biological control of cranefly larvae *Tipula paludosa* (Tipulidae, Diptera). *J.Plant Dis.Prot.*1993; 100(4):343-353. https://www.jstor.org/stable/43386189

45. Peters A, Ehlers RU. Susceptibility of leatherjackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. *J Invertebr Pathol.* 1994;63(2):163-171. DOI:10.1006/jipa.1994.1031.

46. Gazit Y, Rossler Y, Glazer I. Evaluation of entomopathogenic nematodes for the control of Mediterranean fruit fly (Diptera: Tephritidae). *Biocontrol Sci. Technol.* 2000;10: 157-164. https://doi.org/10.1080/09583150029297.

47. Lindegren JE, Wong TT, McInnis DO. Response of Mediterranean fruit fly (Diptera: Tephritidae) to the entomogenous nematode *Steinernema feltiae* infield tests in Hawaii. *Environ.Entomol.*1990; 19: 383-386

48. Chergui S, Benzehra A, Boudjemaa K, Barkou H, Karaca I. Efficacy of Turkish isolate of *Steinernema feltiae* (Rhabditida: Steinernematidae) in controlling the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), under laboratory conditions. *Egypt. J.Biol.Pest Control.* 2019;29: 01-07. https://doi.org/10.1186/s41938-019-0162-4

49. Mokrini F, Laasli SE, Benseddik Y, Joutei AB, Blenzar A, Lakhal H, et al. Potential of Moroccan entomopathogenic nematodes for the control of the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Sci. Rep.* 2020; 10:19204 https://doi.org/10.1038/s41598-020-76170-7

50. Rohde C, Moino AJ, Mat S, Carvalho FD, Ferreira CS. Influence of soil temperature and moisture on the infectivity of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) against larvae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Neotrop. Entomol.* 2010; 39: 608-611. DOI: 10.1590/s1519-566x2010000400022.

51. Rohde C, Mertz NR, Moino AJ. Entomopathogenic nematodes on control of mediterranean fruit fly (Diptera: Tephritidae). *Rev. Caatinga, Mossoró.* 2020; 33(4): 974 - 984. DOI:10.1590/1983-21252020v33n412rc.

52. Jean-Baptiste MC, Lima de Brida A, Bernardi D, da Costa Días S, Pazini JB, Leite LG. et al. Effectiveness of entomopathogenic nematodes against *Ceratitis capitata* (Diptera: Tephritidae) pupae and nematode compatibility with chemical insecticides. *J. Econ. Entomol.* 2021; 114(1): 248-256. https:// doi. Org /10.1093/jee/toaa301

53. James M, Malan AP, Addison P. Surveying and screening south African entomopathogenic nematodes for the control of the mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Crop Prot.*2018;105: 41-48. https://doi.org/10.1016/j.cropro.2017.11.008

54. Nouh GM, Hussein MA. The role of entomopathogenic nematodes as biocontrol agents against some tephritid flies. *ABR.* 2014; 8(6):301-306.

55. Shaurub EH, Soliman N., Hashem AG, Abdel-Rahman AM. Infectivity of four entomopathogenic nematodes in relation to environmental factors and their effects on the biochemistry of the Medfly *Ceratitis capitata* (Wied.)(Diptera: Tephritidae). *Neotrop. Entomol.* 2015;44 (6): 610-618. doi: 10.1007/s13744-015-0332-3.

56. Malan AP, Manrakhan A. Susceptibility of the mediterranean fruit fly (*Ceratitis capitata*) and the natal fruit fly (*Ceratitis rosa*) to entomopathogenic nematodes. *J. Invertebr. Pathol.*2009; 100(1): 47-49 DOI: 10.1016/j.jip.2008.09.007.

57. Karagoz M, Gulcu B, Hazir C, Kaya HK, Hazir S. Biological control potential of Turkish entomopathogenic nematodes against the Mediterranean fruit fly *Ceratitis capitata*. *Phytoparasitica*.2009; 37(2):153-159.

58. Patterson SJE, Lacey LA. Susceptibility of western cherry fruit fly (Diptera: Tephritidae) to five species of entomopathogenic nematodes in laboratory studies. *J.Invertebr. Pathol.*1999; 74(2): 206-208. DOI: 10.1006/jipa.1999.4854.

59. Minas RS, Souza RM, Dolinski C, Carvalho RS, Burla RS. Potential of entomopathogenic nematodes (Rhabditida: Heterorhabditidae) to control Mediterranean fruit fly (Diptera: Tephritidae) soil stages. *Nematoda*. 2016; 3: 1-14. https: //doi. org/ 10.4322/nematoda.02016.

60. Foelkel E, Voss M, Monteiro LB, Nishimura G. Isolation of entomopathogenic nematodes in an apple orchard in Southern Brazil and its virulence to *Anastrepha fraterculus*

(Diptera:Tephritidae) larvae, under laboratory conditions. *Braz.J.Biol.*2017; 77(1):22-28. DOI: 10.1590/1519-6984.08315

61. Barbosa-Negrisoli CRC, Garcia MS, Dolinski C, Negrisoli JR, Bernardi D, Nava DE. Efficacy of indigenous entomopathogenic nematodes (Rhabditidae: Heterorhabditidae, Steinernematidae), from Rio Grande do Sul Brazil, against *Anastrepha fraterculus* Wied (Diptera: Tephritidae) in peach orchards. *J.Invertebr. Pathol.* 2009; 102:6-13 https://doi.org/10.1016/j.jip.2009.05.005
62. Heve WK, Adjadeh TA, Billahc MK. Overview and future research needs for development of effective biocontrol strategies for management of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in sub-Saharan Africa. *Pest Manag. Sci.,* 2021; 77: 4224-4237.https://doi.org/10.1002/ps.6485.
63. Godjo A, Chabi N, Zadji L, Dossou P, Batcho O, Baimey H et al. Evaluation of the ability of indigenous nematode isolates of *Heterorhabditis taysearae* and *Steinernema kandii* to control mango fruitfly *Bactrocera dorsalis* under laboratory, semi-field and field conditions in Northern Benin. *Crop Prot.* 2021; 149, 105754. https://doi.org/10.1016/j.cropro.2021.105754
64. Chen S, Han X, Moens M. Effect of temperature on the pathogenicity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) to *Delia radicum*. *Biocontrol.* 2003; 48:713-724. https://doi.org/10.1023/A:1026341325264
65. Pena JM, Carrillo MA, Hallem EA. Variation in the susceptibility of *Drosophila* to different entomopathogenic nematodes. *Infect. Immun.* 2015; 83 (3): 1130-1138. doi: 10.1128/IAI.02740-14
66. Garriga A, Morton A, Garcia-del-Pino F. Is *Drosophila suzukii* as susceptible to entomopathogenic nematodes as *Drosophila melanogaster*? *J.Pest Sci.* 2018; 91(2):789-98. DOI:10.1007/s10340-017-0920-6
67. Hubner A, Englert C, Herz A. Effect of entomopathogenic nematodes on different developmental stages of *Drosophila suzukii* in and outside fruits. *BioControl.* 2017; 62(5):669-680. DOI: 10.1007/s10526-017-9832-x.
68. Kamali S, Karimi J, Hosseini M, Campos-Herrera R, Duncan LW. Biocontrol potential of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema*

carpocapsae on cucurbit fly, *Dacus ciliates* (Diptera: Tephritidae). *Biocontrol Sci. Technol.* 2013; 23 (11):1307-1323. https:// doi.org /10.1080 /09583157.2013.835790.

69. Kapranas A, Chronopoulou A, Lytra IC, Peters A, Milonasa PG, Papachristos DP. Efficacy and residual activity of commercially available entomopathogenic nematode strains for Mediterranean fruit fly control and their ability to infect infested fruits. *Pest Manag. Sci.* 2021;77: 3964-3969. https://doi.org/10.1002/ps.6416.
70. Kapranas A, Anna Chronopoulou A, Peters A, Antonatos S, Lytra I, Milonas P. et al. Early and off-season biological control of medfly with entomopathogenic nematodes: From laboratory experiments to successful field trials. *Biol.Control*. 2023; 179, 105173. https://doi.org/10.1016/j.biocontrol.2023.105173.
71. Abdel-Razek AS, Abd-Elgawad MMM. Spinosad combined with entomopathogenic nematode for biocontrol of the Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]) on citrus. *Egypt J. Biol. Pest Control.* 2021; 31: 112 https://doi.org/10.1186/s41938-021-00458-7
72. Chaneiko SM, de Brida AL, Bernardi D, Leite LG, Garcia FRM. Biological activity of entomopathogenic nematodes on *Anastrepha fraterculus* (Diptera: Tephritidae). *Biosci. J.* 2021; 37, e37047. https://doi.org/10.14393/BJ-v37n0a2021-53895
73. Elise N , Joe S, Diana S , Nabil N.. *In vitro* susceptibility of the pea leafminer *Liriomyza huidobrensis* pupae to entomopathogenic *Heterorhabditis indica* and *Beauveria bassiana*. *Leban. Sci.J*.2015;16. 19-26.
74. Gava CAT, Paranhos BAJ. Combining the virulent *Beauveria bassiana* (Balsam) Vuillemin LCB289 and nematode strains to control pupae of *Ceratitis capitata* Wiedemann. *Biocontrol Sci. Technol.* 2023; 33: 383-396. DOI:10.1080/09583157.2023.2191300.
75. Yagci M, Tugba Akdeniz Fırat TA, Dolunay-Erdogus F, Sahin M. Virulence of four entomopathogenic nematode against different stages of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Egypt. J. Biol. Pest Control.* 2021;31:126. https://doi.org/10.1186/s41938-021-00472-9
76. Usman M, Wakil W, Shapiro-Ilan DI. Entomopathogenic nematodes as biological control agent against *Bactrocera zonata* and *Bactrocera dorsalis* (Diptera: Tephritidae). *Biol. Control.* 2021; 163, 104706. https://doi.org/10.1016/j.biocontrol.2021.104706.

77. Torrini G, Mazza G, Benvenuti C, Simoncini S, Landi S, Frosinini R. et al. Entomopathogenic nematodes as potential biocontrol agents against *Bactrocera oleae* (Diptera: Tephritidae). *Biocontrol Sci. Technol.* 2020; 30: 909-919. DOI: 10. 1080 /09583157.2020.1775177.

78. Toledo J, Morán-Aceves BM, Ibarra JE, Liedo P. Can Entomopathogenic Nematodes and their symbiotic bacteria suppress fruit fly pests? A Review. *Microorganisms.* 2023. ;11(7):1682. doi: 10.3390 / microorganisms 11071682

79. Taylor DB, Szalanski AL, Adams BJ,Peterson RD. Susceptibility of house fly (Diptera: Muscidae) larvae to entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) . *Environ.Entomol.*1998; 27(6): 1514-1519. https://doi.org/10.1093/ee/27.6.1514.

80. Ta-un P, Ehlers R-U, Nimkingrat P. Effects of soil texture and moisture on the host searching abilities of *Steinernema siamkayai* against *Bactrocera latifrons*. *Nematology*. 2022; 24: 559-570. https://doi.org/10.1163/15685411-bja10151.

81. Stark JEP, Lacey LA Susceptibility of Western fruit fly (Diptera: Tephritidae) to five species of entomopathogenic nematodes in laboratory studies. *J. Invertebr. Pathol.* 1999; 74:206-208. DOI: 10.1006/jipa.1999.4854.

82. Shaurub EH. Review of entomopathogenic fungi and nematodes as biological control agents of tephritid fruit flies: Current status and a future vision. *Entomol. Exp. Appl.* 2023; 171: 17-134. DOI: 10.1111/eea.13244.

83. Heve WK, El-Borai FE, Carrillo D, Duncan LW. Increasing entomopathogenic nematode biodiversity reduces efficacy against the Caribbean fruit fly *Anastrepha suspensa*: interaction with the parasitoid *Diachasmimorpha longicaudata*. *J. Pest Sci.* 2018; 91(2):799-813. DOI:10.1007/s10340-017-0942-0.