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

Comment [D1]: Remove

Comment [D2]: Such like

Comment [D3]: Remove

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/m^2) against the second, third, and fourth instar larvae and at temperatures below 25^{o}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

Comment [D4]: Italicised

Comment [D5]: Source?

Comment [D6]: Source?

Comment [D7]: Separate

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/m^2 against the second and the third instars larvae.

Comment [D8]: Source?

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- 3.0×10^6 S. feltiae / m^2 have been tested to achieve satisfactory control of sciarid infestations.

Comment [D9]: Source?

The phorid fly, *Megaselia halterata* (wood) causes considerable problems for mushrooms. Adults are very strongly attracted to compostor 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 optimal because *D. radicum* larvae are only in the soil for a brief period.

Comment [D10]: Source?

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 instars and adults Baits treated with either S. feltiae or H. megidis provided significantly greater control of the adult population compared tosynthetic chemicals.

Comment [D11]: separate

Among the fruit fly species, the oriental fruit fly, *Bactrocera dorsalis* (Hendel) is an important insect pesto f 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.

Comment [D12]: Source?

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 %	Aatif *et al*.,2019;2023
S. feltiae S. corpocapsae H.bacteriophora	Olive fruitfly pupae (*Bactrocera oleae*)	80%	Torrini et al.,2017
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%	Sirjani *et al*.,2009
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	Aryal et al.,2022
S. feltiae, S.carpocapsae H.bacteriophora	Third larval instar of *Queensland fruit fly*	caused significant	Langford et al.,2013

Comment [D13]: Italicised

Comment [D14]: Italicised

Comment [D15]: Italicised

	(Bactrocera tryoni)	larval mortality	
S. feltiae	peach fruit fly, (*Bactrocera zonata*)	100%	Mahmoud. 2007
H.bacteriophora, *H. megidis*, *H. georgiana*, *H. floridensis*, *H.indica*, *S.carpocapsae*, *S. riobrave*, *S. feltiae*, *S. rarum* , *S. glaseri*	larvae, pupae and adultsof *Bactrocera zonataB.dorsalis*	58.50-93.06%	Usman et al.,2021; Wakil et al.,2022
H.bacteriophora, *H.indica*, *S.carpocapsae*, *S.glaseri*.	3rd larval instar of house fly (*Musca domestica*)	43.3-10%	Bream *et al*.,2018
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*)		Grewal & Richardson, 1993; Harris et al., 1995; Scheepmaker *et al*., 1998; Jagdale *et al*., 2004,2007; Jess *et al*. 2005; Tomalak et al., 2005; Grewal,2007 Grewal & Georgis, 1998; Grewal,2007
Steinernema sp. *S. abbasi* *S. pakistanense* *H. indica*	*Megaselia sandhui*	13–33%	Lamba et al. (2008)
H. bacteriophora, *S. carpocapsae*,	chive maggot (*Bradysia odoriphaga*)		Bai et al., 2016;

Comment [D16]: italicised

Comment [D17]: Italicised

S. feltiae, *H. indica*, *S.longicaudum*			Wu et al.,2017
S. yitgalemense *S. feltiae* *S. jeffreyense* *S. khoisanae* *Steinernema sp.* *H. indica* *H. zealandica* *H. bacteriophora* *H. noenieputensis*	*Bradysia impatiens*	52-87%	Katumanyane (2017)
S. carpocapsae All, *S. feltiae* MG-14 *Heterorhabditis* sp.UK 211	*Liriomyza trifolii* *L.huidobrensis* *L. bryoniue*	24-69% 82%	Hara et al., 1993; LeBeck et al., 1993; Sher et al., 2000; Head &Walters, 2003; Tomalak et al.,2005; Williams & Walters,2000 Williams &Macdonald , 1995
S. riobrave, *S. carpocapsae*, *S. feltiae*	fruit flies (*Rhagoletis indifferens*, *Anastrepha ludens*)		Yee and Lacey, 2003; Toledo et al., 2005
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%	Usman et al.,2020
H. bacteriophora, *H. marelatus*, *S.carpocapsae*, *S. feltiae*	European cherry fruit fly (*Rhagoletis cerasi*)	40-95%	Kepenekci et al., 2015
S.bicornutum, *S. carpocapsae*, *S. carpocapsae China*, *S. feltiae* *H.bacteriophora*	European cherry fruit Fly (*Rhagoletis cerasi)*	54%	Koppler et al.,2003

Comment [D18]: Not referenced

H. bacteriophora UWS1 *H.bacteriophora,* *S. carpocapsae* , *S.feltiae*	Crane fly (*Tipula paludosa*)	28-65%	Ansari & Butt,2012; Oestergaard *et al*., 2006; Simard *et al*., 2006
S.feltiae *S.anomali* *S.affinis*	Cranefly larvae (*Tipula paludosa*)	13-90%	Ehlers & Gerwien, 1993
S.feltiae	Cranefly larvae (*Tipula paludosa, Tipula oleracea*)	L1 susceptible stage	Peters & Ehlers,1994
S. riobrave *S. carpocapsae* *S. feltiae*	Mediterranean fruit fly or medfly (*Ceratitis capitata*)	50-100%	Gazit *et al*., 2000; Lindegren *et al*., 1990 Chergui et al.2019.
S.feltiae-SF-MOR9, *S. feltiae*-SF-MOR10 *H.bacteriophora*-HB-MOR7	Medfly (*Ceratitis capitata*)	80%	Mokrini et al.,2020
Heterorhabditis sp. RSC01 , *S.carpocapsae* ALL	Third-instars of Mediterranean fruit fly, (*Ceratitis capitata*)	26.7- 96.7%	Rohde et al.,2010
S.carpocapsae ALL *H.amazonensis* JPM4	larvae and pupae of Mediterranean fruit fly (*Ceratitis capitata*)	50-80%	Rohde et al.,2020;
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%	Jean-Baptiste et al.,2021
H. noenieputensis, *H. indica* *H. bacteriophora* *S.yirgalemense*	Mediterranean fruit fly or Medfly, (*Ceratitis capitata,Ceratitis rosa)*		James et al.,2018
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%	Nouh &Hussein ,2014
H.bacteriophora AS1, *H. bacteriophora* HP88,	Late third instars of the medfly,	caused significant	Shaurub *et al*.,2015

S.carpocapsae ALL, *S. riobrave* ML29	(*Ceratitis capitata*)	mortality	
H.bacteriophora,H.zealandicaS.khoisanae	Larvae, pupae and adults of (*Ceratitis capitata* , *Ceratitis rosa*)	caused significant mortality	Malan & Manrakhan, 2009
S.weiseri, *S. feltiae* 09-31 *S. carpocapsae* *H. bacteriophora*	Last instar larvae of Mediterranean fruit fly or Medfly, (*Ceratitis capitata)*	50-97%	Karagoz et al.,2009
S. feltiae *S. carpocapsae* *S. riobravis* *H. bacteriophora* *H. marelatus*	Third instars of Western cherry fruit fly (*Rhagoletis indifferens*)	caused significant mortality	Patterson & Lacey, 1999
Heterorhabditis sp. LPP17, *Heterorhabditis* sp. LPP14 *H. baujardi* LPP7	Third instar larvae of *(Ceratitiscapitata*)	90- 98.5%	Minas et al.,2016
S. feltiae	*Anastrepha fraterculus*	75%	Foelkel et al.,2017
H. bacteriophora RS88 *S. riobravae* RS59	South American fruit fly, (*Anastrepha fraterculus*)	20-51.3%	Barbosa-Negrisoli et al.,2009
H.bacteriophora *H.indica* *S.feltiae*	Caribfly (*Anastrepha suspense*)		Heve et al.,2018
H. taysearae Azohoue2 *(=H. sonorensis)* *S. kandii*	Larvae and pupae of mango fruit fly (*Bactrocera dorsalis*)		Godjo et al.,2021.
H.bacteriophora	The cabbage maggot (*Delia radicum*)	Caused higher levels of infection	Sharifi-Far et al.,2018 Chen et al.2003
S. carpocapsae	*Drosophilamelanogaster* larvae	Capable of infecting and killing	Pena et al.,2015
H. bacteriophora, *S. carpocapsae,* *S. feltiae,* *S. kraussei*	spotted wing drosophila (*Drosophila suzukii*)	emergence of flies was significantly reduced.	Garriga et al.,2018 Hübner et al.,2017
H. bacteriophora *S.carpocapsae*	cucurbit fly (*Dacus ciliates*)	12-28%	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.

Comment [D19]: Source?

References

Aatif HM., Afzal A., Idrees A., Mansha MZ., Shahid Hanif CM., Ali Y., Ikram K., Ullah MI., Sarkar SC., Alfarraj S., Li J. 2023. Entomopathogenic nematodes for the control of oriental fruit fly *Bacterocera dorsalis* (Diptera: Tephritidae). Journal of King Saud University–Science. 35:102428. https://doi.org /10.1016/ j.jksus. 2022.102428.

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

Abbas MST.2022. Pathogenicity of entomopathogenic nematodes to dipteran leaf miners, house flies and mushroom flies. *Egyptian Journal of Biological Pest Control. 2:76.* https://doi.org/10.1186/s41938-022-00566-y

Abdel-Razek, A.S.; Abd-Elgawad, M.M.M. **2021.** Spinosad combined with entomopathogenic nematode for biocontrol of the Mediterraneanfruit fly (Ceratitis capitata [Wiedemann]) on citrus. Egypt J. Biol. Pest Control, 31: 112

Ansari MA., Butt TM.2012. Evaluation of entomopathogenic fungi and a nematode against the soil-dwelling stages of the crane fly *Tipula paludosa*. Pest Management Science .68: 1337-1344.

Comment [D20]: Not in the manuscript

Aryal S., Nielsen UN., Sumaya NH., Wilson C., Riegler M. 2022. Virulence, penetration rate and reproductive potential of entomopathogenic nematodes from eastern Australia in Queensland fruit fly, *Bactrocera tryoni*. Biol. Control169, 104871.

Bai GY, Xu H, Fu YQ, Wang XY, Shen GS, Ma HK, Ruan WB .2016. A comparison of novel entomopathogenic nematode application methods for control of the chive gnat, *Bradysia odoriphaga* (Diptera: Sciaridae). J Econ Entoml 109(5): 2006–2013

Barbosa-Negrisoli CRC, Garcia MS, Dolinski C, Negrisoli JR, Bernardi D, Nava DE.2009.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 102:6-13

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

Chaneiko SM., de Brida AL., Bernardi D., Leite LG., Garcia FRM.2021. Biological activity of entomopathogenic nematodes on *Anastrephafraterculus* (Diptera: Tephritidae). Biosci. J., 37, e37047.

Chen S., Han X., Moens M.2003. Effect of temperature on the pathogenicity of entomopathogenic nematodes (Steinernema and Heterorhabditis spp.) to *Delia radicum*. BioControl. 48:713-724

Chergui S., Benzehra A., Boudjemaa K., Barkou H ., Karaca I.2019. Efficacy of Turkish isolate of *Steinernema feltiae* (Rhabditida: Steinernematidae) in controlling the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), under laboratory conditions. Egyptian Journal of Biological Pest Control, 29: 01-07

Ehlers RU., Gerwien A.1993. Selection of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae, Nematoda) for the biological control of cranefly larvae *Tipula paludosa* (Tipulidae, Diptera). Journal of Plant Diseases and Protection. 100(4):343-353.

Elise N ., Joe S., Diana S ., Nabil N. 2015. *In vitro* susceptibility of the pea leafminer *Liriomyza huidobrensis* pupae to entomopathogenic *Heterorhabditis indica* and *Beauveria bassiana*. Lebanese Science Journal. 16. 19-26.

Comment [D21]: Not in the manuscript

Foelkel E, Voss M, Monteiro LB, Nishimura G. 2017. Isolation of entomopathogenic nematodes in an apple orchard in Southern Brazil and its virulence to *Anastrepha fraterculus* (Diptera:Tephritidae) larvae, under laboratory conditions. Brazilian Journal of Biology.77(1):22-28.

Garriga A, Morton A, Garcia-del-Pino F. 2018. Is *Drosophila suzukii* as susceptible to entomopathogenic nematodes as *Drosophila melanogaster* ? Journal of Pest Science. 91(2):789-98.

Gava CAT., Paranhos BAJ.2023. Combining the virulent *Beauveria bassiana* (Balsam) Vuillemin LCB289 and nematode strains to control pupae of *Ceratitis capitata* Wiedemann. Biocontrol Sci. Technol., 33: 383-396

Gazit Y., Rossler Y., Glazer I., 2000. Evaluation of entomopathogenic nematodes for the control of Mediterranean fruit fly (Diptera: Tephritidae). Biocontrol Science and Technology. 10: 157-164.

Godjo A., Chabi N., Zadji L., Dossou P., Batcho O., Baimey H., Bonou W., Sinzogan AAC., Bokonon-Ganta A., Decraemer W. 2021.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., 149, 105754

Grewal PS., Koppenhöfer AM., Choo HY. 2005. Lawn, turfgrass and pasture applications. In: Grewal PS., Ehler RU., Shapiro-Ilan DI. (Eds.), Nematodes As Biocontrol Agents. CABI Publishing, Wallingford, pp. 115-146.

Grewal PS., Richardson PN. 1993. Effects of application rates of *Steinernema feltiae* (Nematoda: Steinernematidae) on control of the mushroom sciarid fly *Lycoriella auripila*. Biocontrol Science and Technology. 3:29-40.

Hara AH., Kaya HK., Gaugler R., LeBeck LM., Mello CL.1993. Entomopathogenic nematodes for biological control of the leafminer, *Liriomyza trifolii* (Dipt. Agromyzidae) (Rhabditida: Steinernematidae). Entomaphaga 38:359-369.

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

Comment [D22]: Not in the manuscript

Comment [D23]: Is it 2007 or 2005

Head, J., Walters, KFA. 2003. 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, 136-140.10.13140/2.1.2799.5528. DOI:10.13140/2.1.2799.5528.

Heve WK, El-Borai FE, Carrillo D, Duncan LW. 2018. Increasing entomopathogenic nematode biodiversity reduces efficacy against the Caribbean fruit fly *Anastrepha suspensa*: interaction with the parasitoid *Diachasmimorpha longicaudata*. Journal of Pest Science. 91(2):799-813.

Heve WK., Adjadeh TA., Billahc MK. 2021.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., 77: 4224-4237

Hubner A, Englert C, Herz A. 2017. Effect of entomopathogenic nematodes on different developmental stages of *Drosophila suzukii* in and outside fruits. BioControl 62(5):669-680.

Hussein MA., El-Wakei N, El-Sebai T. 2006. Susceptibility of melon fruit fly, *Dacus ciliatus* (Loew) (Diptera: Tephritidae) to Entomopathogenic Nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) and to insecticide. Int. J. Nematol. 16(1): 13-18

Jagdale GB., Casey ML., Canas L., Grewal PS. 2007. 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.Biological Control 43:23-30.

Jagdale GB., Casey ML., Grewal PS., Lindquist RK.2004. Application rate and timing, potting medium, and host plant effects on the efficacy of *Steinernema feltiae* against the fungus gnat, *Bradysia coprophila*, in floriculture. Biological Control29:296-305.

James, M., Malan, AP., Addison P. 2018. Surveying and screening south African entomopathogenic nematodes for the control of the mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Crop Protection, 105: 41-48.

Jean-Baptiste MC., Lima de Brida A., Bernardi D., da Costa Días S., Pazini JB., Leite LG., Siciliano-Wilcken SR., Mello-Garcia FR. 2021.Effectiveness of entomopathogenic

Comment [D24]:

Comment [D25]: Not in the manuscript

Comment [D26]: Not in the manuscript

nematodes against *Ceratitis capitata* (Diptera: Tephritidae) pupae and nematode compatibility with chemical insecticides. J. Econ. Entomol., 114(1): 248-256.

Jess S., Schweizer H., Kilpatrick M. 2005. Mushroom applications. In: Grewal PS., Ehler RU., Shapiro-Ilan DI. (Eds.), Nematodes As Biocontrol Agents. CABI Publishing, Wallingford, pp. 191-213.

Kamali S., Karimi J., Hosseini M., Campos-Herrera R., Duncan LW. 2013. Biocontrol potential of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* on cucurbit fly, *Dacus ciliates* (Diptera: Tephritidae). Biocontrol Sci. Tech. 23 (11):1307-1323.

Kapranas A., Anna Chronopoulou A., Peters A., Antonatos S., Lytra I., Milonas P., Papachristos D.2023. Early and off-season biological control of medfly with entomopathogenic nematodes: From laboratory experiments to successful field trials. Biol.Control, 179, 105173

Kapranas A., Chronopoulou A., Lytra IC., Peters A., Milonasa PG., Papachristos DP.2021. 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. 77: 3964-3969

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

Katumanyane A.2017. 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 https:// schol ar. sun. ac. za

Kepenekci I., Hazir S., Ozdem A. 2015. 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. 39(1):74-79.

Koppenhöfer AM. Shapiro-Ilan DI. Hiltpold I.2020. Entomopathogenic nematodes in sustainable food production. Front. Sustain.Food Syst., 4: 125

Comment [D27]: Not in the manuscript

Comment [D28]: Not in the manuscript

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

Lamba JS, Walia KK, Mrig K, Walia RK.2008. Infectivity and virulence of indigenous entomopathogenic nematodes to mushroom phorid fly, *Megaselia sandhui*. J Biol Control 22(2):411-416

Langford EA., Nielsen UN., Johnson SN., Riegler M., 2013. Susceptibility of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), to entomopathogenic nematodes. Bio Control 96: 34-39.

LeBeck LM., Gaugler R., Kaya HK., Hara AH., Johnson MW. 1993. Host stage suitability of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Journal of Invertebrate Pathology. 62:58-63.

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

Mahmoud MF. 2007. 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. 43(1):19-25.

Malan, AP. Manrakhan A. 2009. Susceptibility of the mediterranean fruit fly (*Ceratitis capitata*) and the natal fruit fly (*Ceratitis rosa*) to entomopathogenic nematodes. Journal of Invertebrate Pathology, 100(1): 47-49

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

Mokrini F, Laasli SE, Benseddik Y, Joutei AB, Blenzar A, Lakhal H, Sbaghi M, Imren M, Özer G, Paulitz T, Lahlali R, Dababat AA. 2020. Potential of Moroccan entomopathogenic nematodes for the control of the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Scientific Reports. 10:19204 https://doi.org/10.1038/s41598-020-76170-7

Mullens BA., Meyer JA., Cyr TL.1987. Infectivity of insect-parasitic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) for larvae of some Manure-breeding flies (Diptera: Muscidae). Environ. Entomol., 16: 769-773.

Navarro MJ., Gea FJ.2014. Entomopathogenic nematodes for the control of phorid and sciarid flies in mushroom crops. Pesq.Agropec. Bras.,Brasilia. 49(1): 11-17

Nouh GM, Hussein MA. 2014. The role of entomopathogenic nematodes as biocontrol agents against some tephritid flies. Advances in Biological Research. 8(6):301-306.

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

Patterson Stark JE., Lacey LA. 1999. Susceptibility of western cherry fruit fly (Diptera: Tephritidae) to five species of entomopathogenic nematodes in laboratory studies. Journal of Invertebrate Pathology 74(2): 206-208

Pena JM., Carrillo MA., Hallem EA.2015. Variation in the susceptibility of *Drosophila* to different entomopathogenic nematodes. Infect. Immun. 83 (3): 1130-1138. doi: 10.1128/IAI.02740-14

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

Rohde C, Mertz NR, Moino AJ. 2020. Entomopathogenic nematodes on control of mediterranean fruit fly (Diptera: Tephritidae). Rev. Caatinga, Mossoró, 33(4): 974 - 984.

Rohde C., Moino AJ., Mat S., Carvalho FD., Ferreira CS. 2010. 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. 39: 608-611.

Scheepmaker JWA., Geels FP., Rutjens AJ., Smits PH., Van Griensven LJ.1998. Comparison of the efficacy of entomopathogenic nematodes for the biological control of the mushroom pests *Lycoriella auripila* (Sciaridae) and *Megaselia halterata* (Phoridae). Biocontrol Science and Technology 8:277-287.

Comment [D29]: Not in the manuscript

Comment [D30]: Not in the manuscript

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

Shaurub EH. 2023.Review of entomopathogenic fungi and nematodes as biological control agents of tephritid fruit flies: Current status and a future vision. Entomol. Exp. Appl. 171: 17-134

Shaurub EH., Soliman NA., Hashem AG., Abdel-Rahman AM. 2015. 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 44 (6): 610-618.

Sher RB., Parrella MP., Kaya HK. 2000. Biological Control of the Leafminer *Liriomyza trifolii* (Burgess): Implications for intraguild predation between *Diglyphus begini* Ashmead and *Steinernema carpocapsae* (Weiser). Biological Control 17(2):155-163.

Simard L., Belair G., Gosselin ME., Dionne J. 2006. Virulence of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against *Tipula paludosa* (Diptera: Tipulidae), a turfgrass pest on golf courses. Biocontrol Science and Technology.16:789-801.

Sirjani FO., Lewis EE., Kaya HK.2009. Evaluation of entomopathogenic nematodes against the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). Biological Control .48 :274-280

Soliman NA. 2002. Pathological and biochemical effects of some entomopathogenic nematodes on Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Ph. D. Thesis, Dept. Entomol. Fac. Sci., Cairo Univ.

Soliman NA.2007. Pathogenicity of three entomopathogenic nematodes to the peach fruit fly, *Bacterocera zonata* (Saunders) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Egypt J Biol Pest Cont 17:121-124

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

Comment [D31]: Not in the manuscript

Comment [D32]: Not in the manuscript

Ta-un P., Ehlers R-U., Nimkingrat P.2022. Effects of soil texture and moisture on the host searching abilities of *Steinernema siamkayai* against *Bactrocera latifrons*. Nematology, 24: 559-570

Taylor DB., Szalanski AL., Adams BJ. Peterson RD. 1998. Susceptibility of house fly (Diptera: Muscidae) larvae to entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) . *Environmental Entomology*, 27(6): 1514-1519.

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

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

Torrini G, Mazza G., Benvenuti C., Roversi PF. 2017. Susceptibility of olive fruitfly, *Bactrocera oleae* (Diptera: Tephritidae) pupae to entomopathogenic nematodes. J. Plant Protect Res. 57: 318-320.

Torrini G., Mazza G., Benvenuti C., Simoncini S., Landi S., Frosinini R., Rocchini A., Roversi PF. 2020.Entomopathogenic nematodes as potential biocontrol agents against *Bactrocera oleae* (Diptera: Tephritidae). Biocontrol Sci. Technol. 30: 909-919

Usman M, Gulzar S, Wakil W, Piñero JC, Leskey TC, Nixon LJ, Hofman CO, Wu S., Shapiro-Ilan D. 2020. Potential of entomopathogenic nematodes against the pupal stage of the apple maggot *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae).Journal of Nematology. 52, e2020-79. DOI: 10.21307/jofnem-2020-079

Usman M., Gulzar S., Wakil W.,Wu S., Piñero JC., Leskey TC., Nixon LJ., Oliveira-Hofman C., Toews MD., Shapiro-Ilan D.2020. Virulence of entomopathogenic fungi to *Rhagoletis pomonella* (Diptera: Tephritidae) and interactions with entomopathogenic nematodes. J. Econ. Entomol., 113: 2627-2633.

Usman M., Wakil W., Shapiro-Ilan DI.2021. Entomopathogenic nematodes as biological control agent against *Bactrocera zonata* and *Bactrocera dorsalis* (Diptera: Tephritidae). Biol. Control, 163, 104706. https://doi.org/10.1016/j.biocontrol.2021.104706

Wakil W., Usman M., Piñero JC., Wu S., Toews MD., Shapiro-Ilan DI. 2022.Combined application of entomopathogenic nematodes and fungi against fruit flies, *Bactrocera*

Comment [D33]: Not in the manuscript

Comment [D34]: Not in the manuscript

Comment [D35]: Not in the manuscript

zonata and *B. dorsalis* (Diptera: Tephritidae): Laboratory cups to field study. Pest Manag. Sci. 78: 2779-2791.

Williams EC., Macdonald OC. 1995. Critical factors required by the nematode *Steinernema feltiae* for the control of the leafminers *Liriomyza huidobrensis, Liriomyza bryoniae*and *Chromatomyia syngenesiae*. Annals of Applied Biology 127: 329-341.

Williams EC., Walters KFA. 2000. Foliar application of the entomopathogenic nematode, *Steinernema feltiae* against leafminers on vegetables. Biocontrol Science and Technology 10: 61-70

Wu H., Gong Q., Fan K., Sun R., Xu Y., Zhang K.2017. Synergistic effect of entomopathogenic nematodes and thiamethoxam in controlling *Bradysiaodoriphaga* Yang and Zhang (Diptera: Sciaridae). Biol. Control, 111:53-60.

Yagc M., Tugba Akdeniz Fırat TA., Dolunay-Erdogus F., Sahin M.2021. Virulence of four entomopathogenic nematode against different stages of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Egypt. J. Biol. Pest Control, 31:126

Yee WL., Lacey LA. 2003. Stage-specific mortality of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to three species of *Steinernema* Nematodes. Biological Control 27:349-356.

Comment [D36]: Not in the manuscript