

Review Form 1.6

Journal Name:	Current Journal of Applied Science and Technology
Manuscript Number:	Ms_CJAST_86678
Title of the Manuscript:	Entomopathogenic nematodes: Impacts on non-target Invertebrates
Type of the Article	Review Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

<https://www.journalciast.com/index.php/CJAST/editorial-policy>)

[Review Form 1.6](#)

PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)																					
<p>Compulsory REVISION comments</p>	<p>Entomopathogenic nematodes: Impact on non-target Invertebrates <i>A difficult review for me, the topic being far from my speciality.</i> Main suggestions:</p> <ul style="list-style-type: none"> - to clarify when you mean insects or invertebrates, starting from the title. Is a possible alternative 'invertebrates, mainly insects'? - To modify the 2 tables, the 3rd column becoming the 1st one. The order is not clear: could it be grouped by EPN? Without repeating it on each line? This would make the reading easier, even if a same ref must be cited more than once. For instance: <table border="0" data-bbox="578 520 1216 745"> <tr> <td>EPN</td> <td>Predator of insect pest</td> <td>Insect pest</td> </tr> <tr> <td>Steinernema kraussei</td> <td>Thereseva ...</td> <td>Sawfly</td> </tr> <tr> <td>S. carpocapsae</td> <td>Earwig ...</td> <td></td> </tr> <tr> <td></td> <td>Philonthus ...</td> <td></td> </tr> <tr> <td></td> <td>Carabid beetle</td> <td></td> </tr> <tr> <td></td> <td>Harmonia</td> <td></td> </tr> <tr> <td>S. scapterisci</td> <td>E. Earwig</td> <td></td> </tr> </table> <ul style="list-style-type: none"> - To use the full scientific name by 1st citation, and then vernacular name only when possible e.g. silkworm. - To complete the conclusions with a clear summary: how many species were tested – mainly 3 ones it seems – how many had an effect, on which non-target species (insects, larvae, adults ...), on "useful" species (predators) or "nasty" ones (pests). <p>The text should be entirely checked for typographic mistakes, from space in latin names, to italics in ref and brackets in ref. Some modifications are proposed in the ms (red), but this is not enough.</p>	EPN	Predator of insect pest	Insect pest	Steinernema kraussei	Thereseva ...	Sawfly	S. carpocapsae	Earwig ...			Philonthus ...			Carabid beetle			Harmonia		S. scapterisci	E. Earwig		
EPN	Predator of insect pest	Insect pest																					
Steinernema kraussei	Thereseva ...	Sawfly																					
S. carpocapsae	Earwig ...																						
	Philonthus ...																						
	Carabid beetle																						
	Harmonia																						
S. scapterisci	E. Earwig																						
<p>Minor REVISION comments</p>	<p>Corected ms:</p> <p style="text-align: right;"><u>Review Article</u></p> <p style="text-align: center;">Entomopathogenic nematodes: Impacts on non-target Invertebrates</p> <p><u>Impact of entomopathogenic nematodes on non-target insects</u></p> <p><u>Insects or invertebrates?</u></p> <p>Abstract: Entomopathogenic nematodes are one the important biological control agents against many pestiferous insects. However, during field application of entomopathogenic nematodes the population of other</p>																						

Formatted: Line spacing: single
 Formatted: Font: (Default) Arial, 10 pt, Italic

beneficial insects may have some impact~~ed~~. Many investigations revealed that the observable impact on field populations of non-target invertebrates ~~/insects?~~ has either been small and or undetectable but some studies which have generally been conducted with high dosages of nematodes under laboratory conditions show a wide range of invertebrate susceptibility. This review gives an idea about the impact of entomopathogenic nematodes on non-target invertebrates ?? and precautions during application.

Key words: Bio-control agent, Entomopathogenic nematodes, predators, parasites and parasitoids, insect-pests.

Introduction

The entomopathogenic nematode (EPNs) has gained unprecedented importance as biological control agent against many insect pests. It has been reported from laboratory and field studies that insect from over 17 orders and 135 families are susceptible to ~~entomopathogenic nematode~~EPNs (Ehlers & Peters, 1996; Hominick, 2002). ~~Entomopathogenic nematodes~~They exhibit a symbiotic relationship with certain bacteria, *Photorhabdus* and *Xenorhabdus*: after entering the host insect, pathogenic bacteria are released by the parasite, and due to bacterial infection ~~that~~causes the insect's death (Georgis,1992; Goodrich-Blair & Clarke). The bacterial symbiont is *Photorhabdus* in nematodes of the genus *Heterorhabditis*, and *Xenorhabdus* in nematodes of the genus *Steinernema* ~~under order Rhabditida~~(Dillman & Sternberg, 2012). EPNs are applied in various habitat of the insect pests like soil-dwelling stage, foliar, in cryptic habitats, those pupate in soil or drop in soil for shade (Begley,1990; Trdan et al.,2007; Laznik et al.,2010; Laznik et al.,2011).

Impact of entomopathogenic nematodes on non-target invertebrates

Because of wide host status of several ~~entomopathogenic nematode~~EPN species, there is a serious concern about the population level of non-target invertebrates ?? (Hazir et al., 2003; Kaiser & Heimpel, 2015). This issue was first raised by Howarth in 1991, who outlined evidence for significant non-target impacts from biological control agents. Coote, (2003) reported that ~~a~~deliberately introduced biocontrol agent may also have off-target effects, and potentially harm wildlife. After that many investigations were carried out regarding the safety to non-target organisms and preceded to development of regulatory protocols to ensure their safe usage (Ehlers, 2010). ~~Though Even if~~entomopathogenic nematodes were isolated from naturally infected insects (Peters, 1996), most have been isolated by baiting of soil samples with a susceptible species (e.g. *Galleria mellonella*) (Akhurst and Brooks, 1984). The efficacy of entomopathogenic nematodes for target species is pronounced. Variable responses have been reported with respect to susceptibility of beneficial insects ~~to~~entomopathogenic nematodes (Poinar,1989; Bathon, 2010; Piedra-Buena et al.,2015; Sandhi & Reddy,2019).

The ideas behind the definition of ~~entomopathogenic nematodes~~EPNs being safe for wildlife included the following: they pose no threat to mammals and birds (Boemare et al., 2010); they have minimal adverse effects on above ground non-target invertebrates (Akhurst, 1990; Akhurst & Smith, 2002) and they do not disperse widely in the environment (Downes & Griffin, 1996). For introduction and commercialization of entomopathogenic nematodes as biological control of insect pests in USA, Environmental Protection Agency exempted all entomopathogenic microorganisms ?? from registration except for the exotic species. According to European regulatory body Organisation for Economic Cooperation and Development (OECD), entomopathogenic nematodes were ~~considered~~ safe for wildlife and should not be regulated (Ehlers & Hokkanen, 1996). However, regulations vary in different European countries. *H.bacteriophora* and *S.feltiae* were considered ~~Full latin names by 1st citation; space~~ harmless according to the International Organization for Biological and Integrated Control-West Palaearctic Regional Section (IOBC-WPRS) classification for side effect (Garriga et al., 2019).

The results of some field trials show a moderate influence of ~~entomopathogenic nematodes~~EPNs on non target arthropods if they are used only in short term pest control or even ~~the~~absence of such an effect (Georgis et al., 1991; Lynch &Thomas 2000). Bathon (1996) reports that mortality can be observed among the non-target organisms, only a part of the population was under attack but and local impacts were negligible.

Nguyen and Smart (1991), Nuutinen et al.,_(1991), and (Patil et al.,2016) investigated the impact of different entomopathogenic nematode species on various earthworm species. In some instances, nematode development was reported but no impact on earthworm populations was observed. In soil column tests, improved dispersal of *S.carpocapsae* was observed when earthworm (*Lumbricus terrestris* or *Aporrectodea trapezoids*) were present, nematodes were found present on the exterior and interior of the earthworms (Capinera et al.,1982; Shapiro et al.,1993)

The honey bee, *Apis mellifera* L., is relatively non-susceptible to infection (Cantwell et al., 1972, Hackett and Poinar 1973, Kaya et al., 1982; Baur et al.,(1995) Erler et al.,2022). By using spray applications with *S. carpocapsae*, *Neoaeplectana carpocapsae*, *S. glaseri* it was observed that ~~they had~~no effect on mortality or behavior of *Apis mellifera* (Baur et al., 1995). However, ~~as a direct effect~~,entomopathogenic nematodes applied in the field would contact free ranging bees causing an effect (Hackett & Poinar,1973; Zoltowska et al.,2003; Taha & Abdelmegeed,2016). For *H. bacteriophora* and *S. riobravis*, a concentration-~~dependent~~ increase to high

Formatted: Font: (Default) Arial, 10 pt, Superscript

mortality was detectable for honey bee (*A. mellifera* sp.) brood and adults, with apparent variability for the different species and strains. The same can be seen for the majority of studies using microcolonies and normal size colonies, with no effects on adult bees but increased brood mortality (Younis & Fergani, 2020). Nematode reproduction might also be species-specific or an interaction of the host, its parasite, and the environment (Kaya et al., 1982). Some honey bees even showed to be tolerant towards infections with *H. bacteriophora* HP88, *H. taysera*, or *Heterorhabditis* sp. S1 (Shamseldan et al., 2004). As only few studies used full-size colonies with constant environmental conditions, infectivity has to be tested for the different nematode species on colony level, to see if the observed effects from cage assays are reproducible.

Ishibashi et al., (1987) observed infection of DD-136 define to silkworm *Bombyx mori* and the parasitoid, *Trichomalus apanteloctenus*. Infections were confirmed by reproducing nematodes. *Bombus terrestris* has been tested only by applying two commercial products (contact exposure) in cage assays. In both cases, concentration-dependent high mortality was observed, with bees successfully infected by the nematodes (lower number for *S. kraussei* than for the combination of *Heterorhabditis* sp. and *Steinernema* spp.) (Dutka et al., 2015).

S. bicornutum and *S. feltiae* did not have effect on the larval survival to of the Chinese oak silkworm (*Antheraea pernyi*) and mulberry silkworm (*Bombyx mori*), whereas *S. carpocapsae* and *S. glaseri* did have an effect. Each *Steinernema* species poses no threat to hatchability of eggs, pupation rate, larval durations and not cocoon-to-shell ratio (Dong et al., 2020).

Predators and parasitoids are potentially affected by entomopathogenic nematodes, through direct infection or early death of the parasitized host, or reduction in the host population. Harvey et al., (2016) evaluated the direct non-target effects and indirect non-target effects in a forest ecosystem during inundative application of exotic *S. carpocapsae* and *H. downsi* and native strain of *S. feltiae* to suppress the large pine weevil (*Hyllobius abietis*). The exotic species were accorded a lower overall risk status than native species-ones and strains because of their shorter persistence in the target environment. Gaugler (1981) stated that though *S. feltiae* has wide host range to control cryptic pests, not harmful to beneficial insects as having poor persistence on foliage. In a pot experiment it was found that in *Steinernema carpocapsae* treatment, parasitoids and predator nymphs had a survival rate of up to 76% while, in adult predators, survival ranged from 14% to 100% (Garriga et al., 2019). Predators are more likely to be affected directly by infection (Table 1). Direct treatment of predators with EPNs in laboratory revealed that some species were found to be highly susceptible to infection (Georgis & Hague, 1991; Powell & Webster, 2004; Rojht et al., 2009; Hodson et al., 2011) and others showed no infection (Mracek & Spitzer, 1983; Lopez, 2015). Larvae of five general predatory insects from the families Carabidae, Cicindelidae, and Staphylinidae were found to be somewhat susceptible to nematode infection, although their adult stage was more resistant (Georgis et al. 1991). Farag (2002) reports a high mortality of the larvae of *Coccinella undecimpunctata* caused by *H. tayserae* and *S. carpocapsae* S2 in a laboratory assay, therefore is it not recommended the to use of entomopathogenic nematodes when these predators are present on the plants in high number.

Nematodes can have direct deleterious effects on parasitoid larvae developing within infected hosts, but that the majority of parasitoid larvae are not infected within the host (Table 2). Most parasitoid larvae that succumb to nematode infection are infected as they emerge from the host. Parasitoids cannot complete their development inside or on nematode-infected hosts if parasitism occurs before or early after infection. The parasitoid females may avoid laying eggs in the infected hosts or sometimes cannot discriminate between healthy and infected hosts. Kaya (1978a) observed susceptibility of parasitoids to nematodes, with older endoparasitoids less affected than young parasitoids. Only the caterpillars parasitized by braconid wasps that were exposed to nematodes for 12 and 24-48 h before adult emergence displayed high levels of adult survival. However, braconid larvae were more affected by nematode infection in lepidopterous hosts than were tachinids (Kaya, 1984). Once braconid larvae began cocoon spinning, susceptibility to nematodes was greatly reduced. Wasps in fully formed cocoons were virtually immune to infection (Shannag & Capinera, 2000). The nonporous inner layer of the cocoon serves as a physical barrier to the nematodes. Though parasitoid larvae die when hosts are infected 2-3 days before wasp maturity, this appears to be a result of the death of the insect host and to nutrient depletion. The nematodes *S. carpocapsae* is somewhat compatible with wasp parasitoids for biological control of melonworms, and would be unlikely to totally disrupt biological suppression of melonworm by *C. diaphaniae* wasps.

Conclusion
Hajek and Goettel, (2007) stated that evaluation of entomopathogenic nematodes and other entomopathogens effects on non-target organisms is an important yet relatively neglected area of study. With the increasing interest in use of EPNs for pest control, the potential effects on non-targets are important for pest control. It This should be seriously taken into consideration. A degree of caution may be advisable when deliberately introducing EPNs to the environment as biological pest control agents, until more is known about their effects on beneficial invertebrates. Assessment of the impact of nematodes on predators and parasitoids should be made on all life

Formatted: Left, Line spacing: single

Formatted: Line spacing: single

stages exposed to nematodes of different species or strains along with their ecological studies. Research on the extent and impact of entomopathogenic nematodes on non-target invertebrates in the field, using commercial dosages of the nematodes, is essential to a proper evaluation of their environmental impact. Recommendations should be given by selective use of entomopathogenic nematodes on pest population enabling the predators or parasitoids to survive in untreated part. Newly developed EPN product must be evaluated for biosafety and environmental impact as well as delivery system.

Table 1: Effect of entomopathogenic nematodes on non-target predators of insect pests

Predators of insect pests	Insect pests	Entomopathogenic nematodes	Lab/Field test	Effect on predators	Reference
<i>Thereva handlirschi</i> , <i>T. valida</i> , <i>Rhago notatus</i>	Sawfly, <i>Cephalia abietis</i>	<i>Steinernema kraussei</i>	Lab	Did not infect the larvae of the predators	Mracek & Spitzer, 1983
Earwig, <i>Labidura riparia</i>	<i>Spodoptera littoralis</i>	<i>Heterorhabditis bacteriophora</i> <i>S. carpocapsae</i>	Lab, Field	Only immature stages infected, Adult stages not infected	Georgis et al., 1991
<i>Philonthus</i> sp.	Fly maggot and mite	<i>H. bacteriophora</i> <i>S. carpocapsae</i>	Lab	Adult was less susceptible than the 3rd larval instar	Georgis & Hague, 1991
European earwig, <i>Furcica auricularia</i>	Aphid, scale insects	<i>S. scapterisci</i>	Lab	Not infected	Grewal et al. 1993
Carabid beetle <i>Bembidion properans</i> , <i>Pterostichus cupreus</i>	Pea weevil <i>Sitona lineatus</i>	<i>S. carpocapsae</i>	Field	No effect	Ropek & Jaworska, 1994
<i>Harmonia axyridis</i>	Aphid, scales, psyllids	<i>S. carpocapsae</i>	Lab	causing temporary paralysis and death	Lemire et al., 1996
Ladybugs <i>Coccinella undecimpunctata</i>	Aphid	<i>H. taysarae</i> <i>S. carpocapsae</i> S2	Lab	high mortality of the larvae of the predator	Farag, 2002
<i>Aphidoletis aphidimyza</i>	Aphid	<i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>H. bacteriophora</i>	Lab	9-93% infection	Powell & Webster, 2004
Coleomegilla maculata , <i>Olla v-nigrum</i> , Harmonia axyridis , Coccinella septempunctata	Aphid	H. bacteriophora S. carpocapsae	Lab	Less impact on lady beetle populations	Shapiro-Illan & Cottrell ,2005
<i>Atheta coriaria</i>	Fungus gnats	<i>S. feltiae</i>	Lab	No impact	Jandricic et al., 2006
Twospotted lady beetle, <i>Adalia bipunctata</i> , Lacewing <i>Chrysoperla carnea</i>	Aphids	<i>S. feltiae</i> , <i>S. carpocapsae</i> , <i>H. bacteriophora</i>	Lab	Up to 100% mortality	Rojht, 2007; Rojht et al., 2009
European earwig, <i>Furcica</i>	Aphid, scale insects	<i>S. carpocapsae</i>	Lab	Highly infected(84.3%	Hodson et al. 2011

Review Form 1.6

<i>auricularia</i> <i>Chrysoperla zastrowi</i>	Mealybugs, Aphids, Thrips, Psyllids, Whiteflies	<i>H. bacteriophora</i>	Lab	Mortality) Nematode did not affect egg-hatching and survival of larvae or adults of the predator	Lalitha et al., 2012
<i>Dalotia coriaria</i>	Western flower thrips, <i>Frankliniella occidentalis</i> , Fungus gnats <i>Bradysia</i> spp.	<i>H. bacteriophora</i> <i>S. feltiae</i> <i>S. carpocapsae</i> <i>S. riobrave</i>	Lab	Third instars were more susceptible than the adults	Tourtois & Grieshop, 2015
<i>Macrolophus pygmaeus</i> , <i>Nesidiocoris tenuis</i>	<i>Tuta absoluta</i>	<i>S. carpocapsae</i>	Pot	Not infected	Lopez, 2015
Carabid beetle <i>Calosoma granulatum</i>	Spodoptera frugiperda	<i>H. amazonensis</i> RSC 5, JPM 4	Lab	Safe	Mertz et al., 2015
Green lacewing, <i>Chrysoperla carnea</i> seven spotted lady beetle, <i>Coccinella septempunctata</i>	Pirate bug, <i>Orius albidipennis</i>	<i>S. carpocapsae</i> BA2, Sinai, Egypt, <i>S. carpocapsae</i> S2, Sinai, Egypt, <i>H.sp.</i> (D1), Dina Farmers, <i>S. feltiae</i> , <i>S. carpocapsae</i> All, <i>S. riobraevae</i> , <i>S. scabtarisci</i> , <i>S. glasseri</i> , <i>H. bacteriophora</i> HP88 <i>H. marilatus</i> MAR)	Lab	Should avoid using concentrations above 100 IJs/ml of entomopathogenic nematodes during the peak of <i>C. carnea</i> and <i>C. septempunctata</i>	Metwally et al., 2016
<i>Coccinella septempunctata</i> <i>Chrysoperla carnea</i>	<i>Spodoptera littoralis</i>	<i>Heterorhabditis bacteriophora</i> , <i>Steinernema feltiae</i> and <i>Steinernema carpocapsae</i>	Lab, Semi field	Low mortality	Mona et al., 2018
<i>Coccinella undecimpunctata</i>	Tortoise Beetle, <i>Cassida vittata</i>	<i>H. bacteriophora</i> H88 <i>S. carpocapsae</i> S2	Field	Safe	Anter et al., 2020
Table 2: Effect of entomopathogenic nematodes on non-target parasitoids of insect pests					
Parasitoids of insect pests	Insect pests	Entomopathogenic nematodes	Lab/Field test	Effect on predators	Reference
Braconid larval parasitoid, <i>Apanteles militaris</i>	Armyworm, <i>Pseudaletia unipuncta</i>	<i>Neoaplectana carpocapsae</i> , <i>Heterorhabditis heliothidis</i>	Lab	Deleterious effects on larvae, but not cocoon	Kaya, 1978a; 1978b
Ichneumonid, braconid	Tomato hornworms, cabbage worms	<i>Neoaplectana carpocapsae</i>	Lab	Deleterious effects	Kaya & Hotchkin, 1981
<i>Olesicampe monticola</i>	Larch sawfly <i>Cephalcia lariciphila</i>	<i>N. carpocapsae</i>	Lab	Deleterious effects	Georgis & Hague, 1982
Tachinid parasitoid, <i>Myxecoristops</i> sp.	Sawfly, <i>Cephalcia abietis</i>	<i>Steinernema kraussi</i>	Lab	Deleterious effects	Mracek & Spitzer, 1983
Tachinid parasitoid,	Armyworm	<i>N. carpocapsae</i>	Lab	nematodes were unable to develop	Kaya, 1984

Review Form 1.6

<i>Compsilura concinnata</i>				within tachinid-parasitized hosts after the third day of parasitism by the parasitic insect	
Ichneumonid, <i>Xenoschesis fulvipes</i> , <i>Ctenopelma lucifer</i>	spruce web- spinning sawfly, <i>Cephalcia arvensis</i>	<i>S.feltiae</i>	Field	66% reduction in emergence of <i>X. fulvipes</i>	Battisti ,1994
Tachinid, <i>Ormia deplete</i>	mole cricket, <i>Scapteriscus vicinus</i>	<i>S. scapterisci</i>	Lab	Not effected	Parkman & Frank, 2002
<i>Trichogramma chilonis</i> , <i>T. japonicum</i>	<i>Corcyra cephalonica</i>	<i>H. indica</i>	Lab	did not affect percent emergence	Mohan & Sabir, 2005
<i>Bracon hylobii</i>	<i>Hylobius abietis</i>	<i>S. carpocapsae</i> and <i>H. downesi</i>	Field	did not affect the natural populations	Dillon et al.,2008
<i>Cardiochiles diaphaniae</i>	Melonworm <i>Diaphania hyalinata</i> , Pickleworm <i>D. nitidalis</i>	<i>S.carpocapsae</i>	Lab	Nematodes do not kill all parasitoids, the pupal stage is resistant to infection	Shannag &Capinera, 2000
Eulophid parasitoid wasp <i>Diglyphus begini</i>	leafminer <i>Liriomyza trifolii</i>	<i>S.carpocapsae</i>	Lab	Adult <i>D. begini</i> not susceptible to nematode infection, but avoid ovipositing on nematode-infected larvae. However, the presence of nematodes in mines with wasp eggs decreased the chance of wasp survival to adulthood.	Sher et al.,2000
<i>Bracon hylobii</i>	Large Pine Weevil, <i>Hylobius abietis</i>	<i>H.downesi</i>	Lab	Reduction in cocoon formation,emerging adults are killed	Everard et al., 2009
<i>Trichogramma chilonis</i> , <i>T. japonicum</i>	<i>Corcyra cephalonica</i>	<i>H.bacteriophora</i>	Lab	did not affect percent emergence	Lalitha et al., 2012
<i>Microplitis rufiventris</i>	Cotton Leafworm, <i>Spodoptera littoralis</i>	<i>H.bacteriophora</i> , <i>S.carpocapsae</i>	Lab	Safe	Atwa et al.,2013
braconid , <i>Diachusmimorpha longicaudata</i>	Caribbean fruit fly, <i>Anastrepha suspensa</i>	<i>H. bacteriophora</i>	Field	Did not affect on natural population	Heve et al.,2017
<p>References Akhurst R.J, Brooks WM.1984.The distribution of entomophilic nematodes (Heterorhabditidae and Steinernematidae) in North Carolina. J.Invertebr. Pathol. 44(2):140-145. Akhurst, R., Smith, K., 2002. Regulation and safety. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. CABI, New York, NY, pp. 311-332.</p>					

Formatted: Line spacing: single

Review Form 1.6

	<p>Akhurst, R.J. 1990. Safety to nontarget invertebrates of nematodes of economically important pests. In: Laird, M., Lacey, L.A., Davidson, E.W.(Eds.), Safety of Microbial Insecticides. CRC Press, Boca Raton, FL,pp. 233-240.</p> <p>Anter, MA., Mahmoud MM. El-Hassawy, Abou-Donia SA., Abdelmonem A.E. 2020. Comparison between the effectiveness of certain insecticides and entomopathogenic nematodes against Tortoise Beetle, <i>Cassida vittata</i> (Vill.) in sugar beet fields and their side effects on <i>Coccinella undecimpunctata</i>. Egyptian Academic Journal of Biological Sciences. F. Toxicology & Pest Control. 12(2): 277-287.</p> <p>Atwa, AA., Hegazi, AM., Khafagi, WA. , Abd El-Aziz, GM. 2013. Interaction of the Koinobiont Parasitoid <i>Microplitis rufiventris</i> of the Cotton Leafworm, <i>Spodoptera littoralis</i>, with two entomopathogenic Rhabditids, <i>Heterorhabditis bacteriophora</i> and <i>Steinernema carpocapsae</i>. Journal of Insect Science , 13, 84-91. https://doi.org/10.1673/031.013.8401</p> <p>Bathon H. 1996. Impact of entomopathogenic nematodes on nontarget hosts. Biocontrol Science and Technology,6(3):421-434.</p> <p>Bathon, H. 2010. Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Science and Technology, 6: 421-434. https://doi.org/10.1080/09583159631398.</p> <p>Battisti, A. 1994. Effects of entomopathogenic nematodes on the spruce web-spinning sawfly <i>Cephalcia arvensis</i> panzer and its parasitoids in the field. Biocontrol Science and Technology. 4(1):95-102. https://doi.org/10.1080/09583159409355317.</p> <p>Baur ME., Kaya HK., Peng YS., Jiang J.1995. Nonsusceptibility of the honey bee, <i>Apis mellifera</i> (Hymenoptera: Apidae), to Steinernematid and Heterorhabditid nematodes. Journal of Nematology 27(3):378-381.</p> <p>Begley JW.1990.Efficacy against insects in habitats other than soil In: Entomopathogenic nematodes in biological control(R Gaugler and HK Kaya eds.) pp.215-231.CRC Press, Boca Raton, FL.</p> <p>Boemare NE, Laumond C, Mauleon H. 2010. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. Biocontrol Science and Technology. 6:333-345.https://doi.org/10.1080/09583159631316.</p> <p>Cantwell, GE., Lehnert, T., Fowler. J. 1972. Are biological insecticides harmful to the honey bee? Am. Bee J.112: 255-258.</p> <p>Capinera JL.,Blue SL.,Wheeler GS.1982.Survival of earthworms exposed to <i>Neoaplectana carpocapsae</i> nematodes. Journal of Invertebrate Pathology. 39:419-421.</p> <p>Coote,T,Loeve E (2003).From 61 species to five: endemic tree snails of the Society Islands fall prey to an ill-judged biological control programme. Oryx37(1):91-96.</p> <p>Dillman AR., Sternberg PW. 2012. Entomopathogenic nematodes. Current Biology,22(11):R430-1. DOI:10.1016/j.cub.2012.03.047</p> <p>Dillon, A.B., Moore, C.P., Downes, M.J. and Griffin, C.T. (2008) Managing populations of the Large Pine Weevil, <i>Hyllobius abietis</i> , using bottom-up and top-down approach. Forest Ecological Management, 255: 2634-2642. https://doi.org/10.1016/j.foreco.2008.01.021.</p> <p>Dong H.,Yu Bi.,Wang JL.,Liu YQ.,Wang H.2020.Safety evaluation of four entomopathogenic nematode species against two silkworm species. Entomological Research.50(3):155-162.</p> <p>Downes M, Griffin, C. 1996.Dispersal behavior and transmission strategies of the entomopathogenic nematodes <i>Heterorhabditis</i> and <i>Steinernema</i>. Biocontrol Science and Technology.6(3):347-356.DOI: 10.1080/09583159631325</p> <p>Dutka A., McNulty A., Williamson SM (2015), A new threat to bees? Entomopathogenic nematodes used in biological pest control cause rapid mortality in <i>Bombus terrestris</i>. PeerJ 3:e1413; DOI 10.7717/peerj.1413</p> <p>Ehlers RU, Peters, A. 1996. Entomopathogenic nematodes in biological control: feasibility, perspectives and possible risks. In: HMT Hokkanen and JM Lynch (Eds.). Biological Control: Benefits and Risks.pp.119-135.Cambridge University Press.</p> <p>Ehlers RU., Hokkanen HTM. 1996. Insect biocontrol with non-endemic entomopathogenic nematodes (<i>Steinernema</i> and <i>Heterorhabditis</i> spp.): Conclusions and recommendations of a combined OECD and COST workshop on scientific and regulatory policy issues. Biocontrol Science and Technology 6:295-302.</p> <p>Ehlers RU.2010. Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. Biocontrol Science and Technology 6:303-316. https://doi.org/10.1080/09583159631299</p> <p>Erler S, Eckert JH, Steiner M., Alkassab AT.2022. Impact of microorganisms and entomopathogenic nematodes used for plant protection on solitary and social bee pollinators: Host range, specificity, pathogenicity, toxicity, and effects of experimental parameters. Environmental Pollution 302:119051. doi.org/10.1016/j.envpol.2022. 119051</p>	
--	---	--

Review Form 1.6

Everard, A., Griffin, C.T. and Dillon, A.B. (2009) Competition and intraguild predation between the Braconid Parasitoid *Bracon hylobii* and the Entomopathogenic Nematode *Heterorhabditis downesi*, natural enemies of the Large Pine Weevil, *Hylobius abietis*. Bulletin of Entomological Research. 99:151-161. <https://doi.org/10.1017/S0007485308006287>.

Farag NA. 2002. Impact of two entomopathogenic nematodes on the ladybird, *Coccinella undecimpunctata* and its prey, *Aphis fabae*. Ann Agric Sci. 47:431-443.

Garriga, A.; Morton A.; Garcia-Lopez D. Garcia-del-Pino F.2019. Compatibility of entomopathogenic nematodes with natural enemies for horticultural pest control. Biological Control, 138, 104050. <https://doi.org/10.1016/j.biocontrol.2019.104050>

Gaugler R.1981. Biological control potential of neoplectanid nematodes. J.Nematol.13:241-249.

Georgis R., Kaya HK., Gaugler R.1991. Effect of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. Environmental Entomology 20:815-822.

Georgis R.1992.Present and future prospects for entomopathogenic nematode products. Biocontrol Science and Technology 2:83-99.

Georgis, R., Hague, NGM. (1991) Nematodes as biological insecticides. Pesticide Outlook, 2: 29-32.

Georgis, R., Hague, NGM.1982. Interactions between *Neoplectana carpocapsae* (Nematoda) and *Olesicampe monticola*, a parasitoid of the larch sawfly *Cephalcia lariciphila*. IRCS Medical Science: Microbiology, Parasitology and Infectious Diseases 10(7/8): 617.

Goodrich-Blair H, Clarke DI.2007.Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: two roads to the same destination. Molec. Microbiol. 64:260-268.

Grewal, P.S., Gauglar, R., Kaya, H.K. and Wustly, M. (1993) Infectivity of the Entomopathogenic Nematode, *Steinernema scapterisci* (Nematoda: Steinernematidae). Journal of Invertebrate Pathology, 62: 22-28. <https://doi.org/10.1006/jipa.1993.1069>.

Hackett KJ, Poinar Jr.GO.1973.The ability of *Neoplectana carpocapsae* Weiser (Steinernematidae: Rhabditoidea) to infect adult honey bees (*Apis mellifera*, Apidae: Hymenoptera).American Bee Journal.113:100.

Hackett, K.J., Poinar, Jr. GO. 1973. The ability of *Neoplectana carpocapsae* Weiser (Steinernematidae: Rhabditidae) to infect adult honeybees (*Apis mellifera*, Apidae: Hymenoptera). Am. Bee J. 113: 100.

Hajek AE.,Goettel MS.2007. Guidelines for evaluating effects of entomopathogens on non-target organisms. In:Field Manual of Techniques in Invertebrate Pathology.LA.Lacey and HK.Kaya (Eds.). DOI: [10.1007/978-1-4020-5933-9_40](https://doi.org/10.1007/978-1-4020-5933-9_40) Springer.pp.815-833.

Harvey CD.,Williams CD.,Dillon AB.,Griffin CT.2016.Inundative pest control: How risky is it? A case study using entomopathogenic nematodes in a forest ecosystem. Forest Ecology and Management.380:242-251.

Hazir,S., Kaya HK., Stock SP., Keskin N.2003.Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests.Turk.J.Bil.27:181-202

Heve, W.K., Carrillo, D., Kora, F.E., Dunkan, L.W. 2017. Increasing entomopathogenic nematode biodiversity reduces efficacy against the Caribbean Fruit Fly *Anastrepha suspensa*: Interaction with the Parasitoid *Diachasmimorpha longicaudata*. Journal of Pest Science, 91:799-813. <https://doi.org/10.1007/s10340-017-0942-0>

Hodson, A.K., Friedman, M.L., Wu, L.N., Lewis, E.E. 2011. European Earwig (*Forficula auricularia*) as a novel host for the entomopathogenic nematode *Steinernema carpocapsae*. Journal of Invertebrate Pathology, 107, 60-64. <https://doi.org/10.1016/j.jip.2011.02.004>

Hominick WM. 2002. Biogeography. In: R.Gaugler (Eds.). Entomopathogenic nematology. pp.115-143.Wallingford, UK, CABI Publishing.

Ishibashi, N., Young, F., Nakashima, M., Abiru, C. & Haraguchi, N.1987. Effects of application of DD-136 on silkworm, *Bombyx mori*, predatory insect, *Agriophodorus dorni*, parasitoid, *Trichomalus apanteloctenus*, soil mites, and other non-target soil arthropods, with brief notes on feeding behavior and predatory pressure of soil mites, tardigrades, and predatory nematodes on DD-136. In: Recent Advances in Biological Control of Insect Pests by Entomopathogenic Nematodes in Japan.(ISHIBASHI, N., ed.), Ministry of Education, Culture and Science, Saga University, 158-164

Jandricic, S., Scott-Dupree, C.D., Broadbent, A.B., Harris, C.R. and Murphy, G.(2006) Compatibility of *Atheta coriaria* with other biological control agents and reduced-risk insecticides used in greenhouse floriculture integrated pest management Programs for Fungus Gnats. The Canadian Entomologist, 138(5): 712-722.<https://doi.org/10.4039/n05-106>

[Kaiser JM, Heimpel GE. \(2015\). Linking risk and efficacy in biological control host-parasitoid models. Biological Control, 90: 49-60. DOI:10.1016/j.biocontrol.2015.05.005](#)

Kaya, H. K. 1978a. Interaction between *Neoplectana carpocapsae* (Nematoda: Steinernematidae) and

	<p><i>Apanteles militaris</i> (Hymenoptera: Braconidae), a parasitoid of the armyworm, <i>Pseudaletia unipuncta</i>. J. Invertebr. Pathol. 13: 358-364.</p> <p>Kaya, H.K.1984. Effect of the entomogenous nematode <i>Neoaplectana carpocapsae</i> on the Tachinid Parasite <i>Compsilura concinnata</i> (Diptera: Tachinidae). Journal of Nematology, 16: 9-13.</p> <p>Kaya, HK. 1978b. Infectivity of <i>Neoaplectana carpocapsae</i> and <i>Heterorhabditis heliothidis</i> to pupae of parasite <i>Apanteles militaris</i>. J. Nematol. 10: 241-244.</p> <p>Kaya, HK., Hotchkin PG.1981. The nematode <i>Neoaplectana carpocapsae</i> Weiser and its effect on selected ichneumonid and braconid parasites. Environ. Entomol.10: 474-478.</p> <p>Kaya, HK., Marston JM., Lindegren JE., Peng Y.S..1982. Low susceptibility of the honey bee, <i>Apis mellifera</i> L. (Hymenoptera: Apidae), to the entomogenous nematode, <i>Neoaplectana carpocapsae</i> Weiser. Environ. Entomol.11: 920-924.</p> <p>Lacey, L., Unruh, T.R. and Headrick, H.L. (2003) Interactions of Two Idiobiont Parasitoids (Hymenoptera: Ichneumonidae) of Codling Moth (Lepidoptera: Tortricidae) with the Entomopathogenic Nematode <i>Steinernema carpocapsae</i> (Rhabditida: Steinernematidae). Journal of Invertebrate Pathology, 83, 230-239. https://doi.org/10.1016/S0022-2011(03)00102-2</p> <p>Lalitha Y.,Nagesh M.,Jalali SK.(2012).Intraguild predation and biosafety of entomopathogenic nematode, <i>Heterorhabditis bacteriophora</i> Poinar et al., and its bacterial symbiont, <i>Photobacterium luminescens</i>, to parasitoid, <i>Trichogramma chilonis</i> Ishii and Predator <i>Chrysoperla zastrowi sillemi</i> (Esben,Petersen). Journal of Biological Control.26: 334-340.</p> <p>Laznik, Z., Toth T., Lakatos T., Vidrih M., Trdan S. (2010). Control of the Colorado potato beetle (<i>Leptinotarsa decemlineata</i> [Say]) on potato under field conditions: a comparison of the efficacy of foliar application of two strains of <i>Steinernema feltiae</i> (Filipjev) and spraying with thiametoxam. Journal of Plant Diseases and Protection, 117: 129-135.</p> <p>Laznik, Z.; Znidarcic DS., Trdan S 2011. Control of <i>Trialeurodes vaporariorum</i> (Westwood) adults on glasshouse-grown cucumbers in four different growth substrates: an efficacy comparison of foliar application of <i>Steinernema feltiae</i> (Filipjev) and spraying with thiamethoxam. Turkish Journal of Agriculture and Forestry, 35:631-640.</p> <p>Lemire S.,Coderre D.,Vincent C.,Belair G.1996.Lethal and sublethal effects of the entomogenous nematode, <i>Steinernema carpocapsae</i>, on the coccinellid <i>Harmonia axyridis</i>. Nematropica 26:284-285.</p> <p>Lopez, D.G. (2015) Susceptibility of predators and parasitoids used in biological control of <i>Tuta absoluta</i> to foliar application of <i>Steinernema carpocapsae</i>. Journal https://www.researchgate.net/publication/281742246</p> <p>Lynch LD,Thomas MB.2000.Non target effects in the biocontrol of insects with insects, nematodes and microbial agents: The evidence. Biocontrol News and Information 21:117-130.</p> <p>Mertz, NR., Sales, FS., Agudelo, AJ., Junior, AM. 2015. Effects of entomopathogenic nematodes on the predator, <i>Calosoma granulatum</i> in the laboratory. Journal of Insect Behavior, 28, 312-327. https://doi.org/10.1007/s10905-015-9503-7</p> <p>Metwally HM., Sabry AH., Gaber NM. 2016. Biosafety of different entomopathogenic nematodes species on some insects natural enemies. Research Journal of Pharmaceutical, Biological and Chemical Sciences.7(6):1878-1883</p> <p>Mohan S, Sabir N.2005. Biosafety concerns on the use of <i>Photobacterium luminescens</i> as biopesticide: experimental evidence of mortality in egg parasitoid <i>Trichogramma</i> spp. Current Science. 89(7):1268-1272.</p> <p>Mona B. El-Mandarawy Basma A. Mahmoud., Gehan M. Nouh.2018. Laboratory and semi-field evaluation of three entomopathogenic nematode species on two non-target insect predators, <i>Coccinella septempunctata</i> (Coleoptera: Coccinellidae) and <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae).Archives of Phytopathology and Plant Protection.51(7-8):423-431. https://doi.org/10.1080/03235408.2018.1477410.</p> <p>Mracek, Z. and Spitzer, K. (1983) Interaction of predators and parasitoids of the Sawfly, <i>Cephalcia abietis</i> (Pamphilidae: Hymenoptera) with its nematode <i>Steinernema kraussei</i>. Journal of Invertebrate Pathology, 42: 397-399. https://doi.org/10.1016/0022-2011(83)90181-7.</p> <p>Nguyen KB,Smart GC,Jr.1991.Pathogenicity of <i>Steinernema scapterisci</i> to selected invertebrates. Journal of Nematology.23:7-11.</p> <p>Nuutinen V., Tyni-Juslin J., Vanninen I., Vainio A.1991.The effects of four entomopathogenic fungi and an entomoparasitic nematode on the hatching of earthworm (<i>Aporrecto caliginosa</i>) cocoons in laboratory. Journal of Insect Pathology 58:147-19.</p> <p>Parkman, J.P. and Frank, J.H. (2002) Interactions between <i>Ormia depleta</i> (Diptera: Tachinidae) and <i>Steinernema scapterisci</i> (Nematoda: Steinernematidae), natural enemies of pest Mole Crickets (Orthoptera: Gryllotalpidae). Environmental Entomology, 1:1226-1230. https://doi.org/10.1603/0046-</p>	
--	--	--

Review Form 1.6

	<p>225X-31.6.1226</p> <p>Patil J., Rajkumar., Subhaharan K.2016.Survival of earthworms exposed to the entomopathogenic nematodes <i>Steinernema carpocapsae</i> and <i>Heterorhabditis indica</i>. Indian J Nematol. 46(1):33-39.</p> <p>Peters A. 1996.The natural host range of <i>Steinernema</i> and <i>Heterorhabditis</i> spp. and their impact on insect populations. Biocontrol Science & Technology6:389-402.</p> <p>Piedra-Buena A, Lopez-Cepero J, Campos-Herrera R. 2015. Entomopathogenic Nematode Production and Application: Regulation, Ecological Impact and Non-target Effects. In book: Nematode Pathogenesis of Insects and Other Pests (Editor) Raquel Campos-Herrera.DOI: 10.1007/978-3-319-18266-7_10.pp.255-282. Springer .</p> <p>Poinar GO.Jr.1989.Non-insect hosts for the entomogenous rhabditoid nematodes <i>Neoaplectana</i> (Steinernematidae) and <i>Heterorhabditis</i> (Heterorhabditidae). Revue de Nematologie.12:423-428.</p> <p>Powell JR, Webster JM.2004. Interguild antagonism between biological controls: impact of entomopathogenic nematode application on an aphid predator, <i>Aphidoletes aphidimyza</i> (Diptera: Cecidomyiidae). Biological Control. 30(1): 110-118. https://doi.org/10.1016/j.biocontrol.2003.09.006.</p> <p>Rojht H, Kac M, Trdan S. 2009. Nontarget effect of entomopathogenic nematodes on larvae of twospotted lady beetle (Coleoptera: Coccinellidae) and green lacewing (Neuroptera: Chrysopidae) under laboratory conditions. J Econ Entomol. 102(4):1440-1443. https://doi.org/10.1603/029.102.0406</p> <p>Rojht, H. 2007. Non-target effect of entomopathogenic nematodes (Rhabditida) against selected predators of aphids (Aphididae). M.S. thesis, University of Ljubljana, Biotechnology Faculty, Ljubljana, Slovenia.</p> <p>Ropek D, Jaworska M.1994. Effect of the entomopathogenic nematode <i>Steinernema carpocapsae</i> Weiser (Nematoda, Steinernematidae) on carabid beetles in field trials with annual legumes. Anz. Schadlingskde, Pflanzenschutz,Umweltschutz, 67(5):97-100</p> <p>Sandhi,R., Reddy GVP.2019. Effects of entomopathogenic nematodes and symbiotic bacteria on non-target arthropods. In book: Microbes for Sustainable Insect Pest Management, Springer. DOI: 10.1007/978-3-030-23045-6_9</p> <p>Shamseldean MM, El-Sadawy E, Allam SFM. 2004. Comparative safety of entomopathogenic nematodes on honeybee workers. Egypt J. Biol. Pest Control.14:147-153.</p> <p>Shannag HK, Capinera JL, 2000. Interference of <i>Steinernema carpocapsae</i> (Nematoda: Steinernematidae) with <i>Cardiochiles diaphaniae</i> (Hymenoptera: Braconidae), a Parasitoid of Melonworm and Pickleworm (Lepidoptera: Pyralidae), <i>Environmental Entomology</i>, 29(3) 612-617. https://doi.org/10.1603/0046-225X-29.3.612</p> <p>Shapiro DI., Berry EC., Lewis LC.1993.Interactions between nematodes and earthworms: enhanced dispersal of <i>Steinernema carpocapsae</i>. Journal of Nematology.25:189-192.</p> <p>Shapiro-Ilan DI, Cottrell TE. 2005. Susceptibility of lady beetles (Coleoptera: Coccinellidae) to entomopathogenic nematodes. J Invertebr Pathol. 89:150-156. https://doi.org/10.1016/j.jip.2005.04.002.</p> <p>Sher RB, Parrella MP, Kaya HK. 2000. Biological control of the leafminer <i>Liriomyza trifolii</i> (Burgess): Implications for intraguild predation between <i>Diglyphus begini</i> Ashmead and <i>Steinernema carpocapsae</i> (Weiser). Biological Control. 17(2): 155-163. https://doi.org/10.1006/bcon.1999.0794</p> <p>Taha EH, Abdelmegeed SM.2016. Effect of entomopathogenic nematodes <i>Heterorhabditis bacteriophora</i> on <i>Galleria mellonella</i> in bee hives of <i>Apis mellifera</i>.J.Biol.Sci.16: 197-201. 10.3923/jbs.2016.197.201.</p> <p>Tourtois, J. and Grieshop, M.J. (2015) Susceptibility of <i>Dalotia coriaria</i> (Kraatz) (Coleoptera: Staphylinidae) to Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae and Steinernematidae). Insects, 6(1): 224-235. https://doi.org/10.3390/insects6010224</p> <p>Trdan, S.; Znidarcic D. Vidrih M. 2007. Control of <i>Frankliniella occidentalis</i> on glasshouse-grown cucumbers: an efficacy comparison of foliar application of <i>Steinernema feltiae</i> and spraying with abamectin. Russian Journal of Nematology, 15: 25-34</p> <p>Wang, Y., Crocker, RL., Wilson, LT. , Smart, G., Wei, X. , Nailon, WT. Jr., Cobb. PP.2001. Effect of nematode and fungal treatments on nontarget turfgrass-inhabiting arthropod and nematode populations. Environ. Entomol. 30(2): 196-203.</p> <p>Younis MS, Fergani YA. 2020. Is it possible to use entomopathogenic nematodes inside honey bee, (<i>Apis mellifera</i> L.) colonies? Egyptian Academic Journal of Biological Sciences,13(1):25-31.</p> <p>Zoltowska, K., Lipinski Z., L.S., Lopienska E. 2003. Beneficial nematodes: a potential threat to honey bees? Bee World 84(3): 125-129.</p>	
<p>Optional/General comments</p>	<p>See higher</p>	

[Review Form 1.6](#)

PART 2:

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

Reviewer Details:

Name:	Joiris CI
Department, University & Country	Belgium