Review Article

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Entomopathogenic nematodes: Impacts on non-target Invertebrates

Impact of entomopathogenic nematodes on non-target insects

Insects or invertebrates?

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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 beneficial insects may have some impacted. 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 nematodeEPNs (Ehlers & Peters, 1996; Hominick, 2002). Entomopathogenic nematodesThey 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).

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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*, *Neoaplectana 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 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 (Shamseldean 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, popupation rate, larval durations ~~and~~ nor 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.downesi* and native strain of *S.feltiae* to suppress the large pine weevil (*Hylobius 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.taysearae* 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

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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 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
Thereva handlirschi, T. valida,	Ssawfly, *Cephalea abietis*	*Steinernema kraussei*	Lab	Ddid not infect the larvae of the	Mracek & Spitzer,

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Rhagio notatus				predators	1983
Eearwig, *Labidura riparia*	*Spodoptera littoralis*	*Heterorhabditis bacteriophora* *S. carpocapsae*	Lab, Field	Only immature stages infected, Adult stages not infected	Georgis et al.,1991
Philonthus sp.	Fly maggot and mite	*H. bacteriophora* *S. carpocapsae*	Lab	Aadult was less susceptible than the 3rd larval instar	Georgis & Hague, 1991
European earwig, *Furficula auricularia*	Aphid,scale insects	*S. scapterisci*	Lab	Not infected	Grewal et al.1993
Carabid beetle *Bembidion properans, Pterostichus cupreus*	Pea weevil *Sitona lineatus*	*S.carpocapsae*	Field	No effect	Ropek & Jaworska, 1994
Harmonia axyridis	Aphid,*scales, psyllids*	*S.carpocapsae*	Lab	causing temporary paralysis and death	Lemire et al., 1996
Ladybugs *Coccinella undecimpunctata*	Aphid	*H.taysearae* *S.carpocapsae* S2	Lab	high mortality of the larvae of the predator	Farag, 2002
Aphidoletis aphidimyza	Aphid	*S. carpocapsae, S.feltiae, H.bacterioph*	Lab	9-93% infection	Powell & Webster, 2004

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Coleomegilla maculata , *Olla v-nigrum,* Harmonia axyridis , Coccinella septempunctata	Aphid	H.bacteriophora S.carpocapsae	Lab	L~~l~~ess impact on lady beetle populations	Shapiro-Ilan & Cottrell ,2005
Atheta coriaria	Fungus gnats	*S. feltiae*	Lab	No impact	Jandricic et al.,2006
Twospotted lady beetle, *Adalia bipunctata,* Lacewing *Chrysoperla carnea*	Aphids	*S. feltiae, S. carpocapsae, H.bacteriophora*	Lab	Up to 100% mortality	Rojht, 2007; Rojht *et al*., 2009
European earwig, *Furficula auricularia*	Aphid,scale insects	*S. carpocapsae*	Lab	Highly infected(84.3% Mortality)	Hodson et al.2011
Chrysoperla zastrowi	Mealybugs,Aphids, Thrips, Psyllids, Whiteflies	*H. bacteriophora*	Lab	Nematode did not affect egg-hatching and survival of larvae or adults of the predator	Lalitha et al., 2012
Dalotia coriaria	Western flower	*H. bacteriophora*	Lab	Third instars were more	Tourtois &

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	thrips, *Frankliniella occidentalis*, Fungus gnats *Bradysia* spp.	*S. feltiae* *S. carpocapsae* *S.riobrave*		susceptible than the adults	Grieshop, 2015
Macrolophus pygmaeus , *Nesidiocoris tenuis*	*Tuta absoluta*	*S. carpocapsae*	Pot	Not infected	Lopez, 2015
Ccarabid beetle Calosoma granulatum	Spodoptera frugiperda	H. amazonensis RSC 5 , JPM 4	Lab	Safe	Mertz et al.,2015
Ggreen lacewing, *Chrysoperla carnea* seven spotted lady beetle, *Coccinella septempunctata*	Ppirate bug, *Orius albidipennis*	*S. carpocapsae* BA2, Sinai, Egypt, *S. carpocapsae* S2, Sinai, Egypt, *H.*sp. (D1),Dina Farmers, *S.feltiae* , *S. carpocapsae* All, *S. riobravae* , *S. scabtarsci* ,	Lab	Should avoid using concentrations above 100 IJs/ml of entomopathogenic nematodes during the peak of *C. carnea* and *C. septempunctata*	Metwally et al.,2016

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		S. glasseri , *H. bacteriophora* HP88 *H. marilatus* MAR)			
Coccinella septumpunctata *Chrysoperla carnea*	*Spodoptera littoralis*	*Heterorhabditis bacteriophora* , *Steinernema feltiae* and *Steinernema carpocapsae*	Lab, Semi field	Llow mortality	Mona et al.,2018
Coccinella undecimpunctata	Tortoise Beetle, *Cassida vittata*	*H.bacteriophora* H88 *S.carpocapsae* 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
Bbraconid larval parasitoid, *Apanteles militaris*	Aarmyworm, *Pseudaletia unipuncta*	*Neoaplictana carpocapsae, Heterorhabditis heliothidis*	Lab	Deleterious effects on larvae, but not cocoon	Kaya ,1978a; 1978b
Ichneumonid ,braconid	Tomato hornworms, cabbageworms	*Neoaplectana carpocapsae*	Lab	Deleterious effects	Kaya & Hotchkin, 1981

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Olesicampe monticola	Llarch sawfly *Cephalcia lariciphila*	*N. carpocapsae*	Lab	Deleterious effects	Georgis & Hague, 1982
Tachinid parasitoid, *Myxecoristops* sp.	Ssawfly, *Cephaleia abietis*	*Steinernema kraussi*	Lab	Deleterious effects	Mracek &Spitzer,1983
Tachinid parasitoid, *Compsilura concinnata*	Aarmyworm	*N. carpocapsae*	Lab	nematodes were unable to develop within tachinid-parasitized hosts after the third day of parasitism by the parasitic insect	Kaya, 1984
Ichneumonid, *Xenoschesis fulvipes, Ctenopelma lucifer*	spruce web- spinning sawfly, *Cephalcia arvensis*	*S.feltiae*	Field	66% reduction in emergence of *X. fulvipes*	Battisti ,1994
Tachinid, *Ormia deplete*	mole cricket, *Scapteriscus vicinus*	*S. scapterisci*	Lab	Not effected	Parkman & Frank, 2002
Trichogramma chilonis,	*Corcyra cephalonica*	*H. indica*	Lab	did not affect	Mohan &

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T. japonicum				percent emergence	Sabir, 2005
Bracon hylobii	*Hylobius abietis*	*S. carpocapsae* and *H. downesi*	Field	did not affect the natural populations	Dillon et al.,2008
Cardiochiles diaphaniae	Melonworm *Diaphania hyalinata* , Pickleworm *D. nitidalis*	*S.carpocapsae*	Lab	Nematodes do not kill all parasitoids, the pupal stage is resistant to infection	Shannag &Capinera, 2000
Eulophid parasitoid wasp *Diglyphus begini*	leafminer *Liriomyza trifolii*	*S.carpocapsae*	Lab	Adult *D. begini* not susceptible to nematode infection, but avoid ovipositing on nematode-infected larvae. However, the presence of nematodes in mines with wasp eggs decreased	Sher et al.,2000

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				the chance of wasp survival to adulthood.	
Bracon hylobii	Large Pine Weevil, *Hylobius abietis*	*H.downesi*	Lab	Reduction in cocoon formation,emerging adults are killed	Everard et al., 2009
Trichogramma chilonis, *T. japonicum*	*Corcyra cephalonica*	*H.bacteriophora*	Lab	did not affect percent emergence	Lalitha et al., 2012
Microplitis rufiventris	Cotton Leafworm, *Spodoptera littoralis*	*H.bacteriophora*, *S.carpocapsae*	Lab	Safe	Atwa et al.,2013
braconid , *Diachusmimorpha longicaudata*	Caribbean fruit fly, *Anastrepha suspensa*	*H. bacteriophora*	Field	Did not affect on natural population	Heve et al.,2017

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