Entomopathogenic nematodes: Impacts on non-target Invertebrates

Abstract: Entomopathogenic nematodes are one the important biological control agent against many pestiferous insects. However, during field application of entomopathogenic nematodes the population of other beneficial insects may have some impact. Though under laboratory condition with high doses of infective juvenile of entomopathogenic nematodes shows wide range of susceptibility to non target invertebrates, but under field conditions the impact on non-target invertebrates has either been small and or undetectable .This review gives an idea about the susceptibility and their impact of entomopathogenic nematodes on non-target invertebrates and precautions to be taken during application.

Key words: Bio-control agent, Entomopathogenic nematodes, predators, parasite and parasitoid, insect-pests.

Introduction

The entomopathogenic nematode (EPNs) specifically *Steinernema* and *Heterorhabditis* genera under order Rhabditida, has gained unprecedented importance as biological control agent against many insect pests [1]. Laboratory and field studies revealed that insect from over 17 orders and 135 families are susceptible to entomopathogenic nematodes [2, 3]. These nematodes exhibit a symbiotic relationship with bacteria *Photorhabdus* in case of *Heterorhabditis* and *Xenorhabdus* in case of *Steinernema*: after entering the host insect, pathogenic bacteria are released by the parasite, cause septicemia that causes the death of the insect [4, 5] . 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 [6-9] .

Impact of entomopathogenic nematodes on non-target invertebrates

Because of wide host status of several entomopathogenic nematode species, there is a serious concern about the population level of non-target invertebrates especially beneficial insects [10,11]. Howarth in 1991, for the first time showed evidence for significant non-target susceptibility. Later on Coote & Loeve [12] reported that intentionally introduced biocontrol agent may also have harmful off-target effects. Many investigations were carried out for the safety of non-target insects and preceded to development of regulatory protocols to ensure the safe usage of biocontrol agents [13]. The entomopathogenic nematodes can be isolated from naturally infected insects [14] ,but most have been isolated by baiting of soil samples with a susceptible species (e.g. *Galleria mellonella*) [15] .The efficacy of entomopathogenic nematodes for target species is well-defined. Many workers reported variable responses with respect to susceptibility of beneficial insects to entomopathogenic nematodes [16-19]. The advantages of application of entomopathogenic nematodes included the following: they pose no threat to mammals and birds [20], they have minimal adverse effects on above ground non-target invertebrates [21, 22] and they do not disperse widely in the environment [23]. For 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 Organization for Economic Cooperation and Development (OECD), entomopathogenic nematodes were safe for beneficial insects and should not be regulated [24] . However, regulations vary in different European countries. Garriga *et al.*, [25] stated that *H.bacteriophora* and *S.feltiae* were considered harmless according to the International Organization for Biological and Integrated Control-West Palaearctic Regional Section (IOBC-WPRS) classification for side effect.

The results of some field trials show a moderate influence or even absence of entomopathogenic nematodes on non target arthropods if they are applied only in short term pest control [26,27]. Bathon [28] reported that mortality can be observed only a part of the population among the non-target organisms.

The impact of different entomopathogenic nematode species on various earthworm species was investigated. In some instances, nematode development was reported but no impact on earthworm populations was observed [29-31] .In soil column tests, dispersal of *S.carpocapsae* was observed on earthworm (*Lumbricus terrestris* or *Aporrectodea trapezoids*), and nematodes were found present on both exterior and interior of the earthworm body [32,33]. Cantwell *et al*., [34]; Hackett & Poinar [35]; Kaya *et al*., [36]; Baur *et al*.,[37]; Erler *et al*.,[38] during their observation on effect of entomopathogenic nematodes (*S. carpocapsae*, *Neoaplectana carpocapsae*, *S. glaseri*) on honey bee, *Apis mellifera* L. However, entomopathogenic nematodes applied in the field through direct contact of free ranging bees causing mortality (Hackett & Poinar,[35]; Zoltowska *et al*.,[39]; Taha & Abdelmegeed,[40] . *H. bacteriophora* and *S. riobravis*, cause high mortality to *A. mellifera* brood and adults, which depend upon high dose as well as species and strains [36]. Younis & Fergani, [41] observed the same result using microcolonies and normal size colonies. Some honey bees showed to be tolerant towards infections with *H. bacteriophora* HP88, *H. taysera*, or *Heterorhabditis* sp. S1 [42].

The silkworm *Bombyx mori* was infected by DD-136 [43].Nematodes were found to be reproduced on silkworm larvae. Dutka *et al*., [44] observed high mortality of *Bombus terrestris* with *S. kraussei* and the combination of *Heterorhabditis* sp. and *Steinernema* sp. in cage assays. Dong *et al*., [45] observed that *S.bicornutum* and *S.feltiae* did not have effect on the larval survival to 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 cocoon shell ratio.

Predators and parasitoids are natural enemies of pestiferous insects. And are considered as important biocontrol agents. These natural enemies may be infected by entomopathogenic nematodes, through direct infection or early death of the parasitized host, or reduction in the host population. Harvey *et al*., [46] evaluated the direct non-target effects and indirect non target effects during inundative application of exotic *S.carpocapsae* and *H.downesi* and native strain of *S.feltiae* to suppress the large pine weevil (*Hylobius abietis*) in a forest ecosystem. The exotic species were accorded a lower overall risk status than native species and strains because of their shorter persistence in the target environment. Gaugler [47] stated that though *S.feltiae* has wide host range to control cryptic pests, not harmful to beneficial insects as having poor persistence on foliage. Garriga *et al*.,[25] found that in *S. carpocapsae* treatment, parasitoids and predator nymphs had a survival rate of up to 76% while, in adult predators, survival ranged from 14% to 100% in a pot experiment.

Predators from the families Carabidae, Cicindelidae, and Staphylinidae were found to be more susceptible to nematode infection, although their adult stage was more resistant[26] and they are more likely to be affected directly by infection (Table 1). Georgis & Hague, [48]; Powell & Webster, [49]; Rojht *et al*., [50]; Hodson *et al*., [51],observed high mortality by direct treatment with EPNs. Mracek & Spitzer, [52]; Lopez, [53] showed no infection. Farag [54] reported a high mortality of the larvae of *Coccinella undecimpunctata* caused by *H.taysearae* and *S.carpocapsae* S2 in a laboratory assay,

Table 1: Effect of entomopathogenic nematodes on nontarget predator of insect pests

Predators of	Insect pests	Entomopathogenic	Lab/	Effect on	Reference

insect pests		nematodes	Field test	predators	
Thereva handlirschi, T. valida, Rhagio notatus	Sawfly, *Cephaleia abietis*	*Steinernema kraussei*	Lab	Did not infect the larvae of the predators	[52]
Earwig, *Labidura riparia*	*Spodoptera littoralis*	*Heterorhabditis bacteriophora S. carpocapsae*	Lab, Field	Only immature stages infected, Adult stages not infected	[26]
Philonthus sp.	Fly maggot, mite	*H. bacteriophora S. carpocapsae*	Lab	Adult was less susceptible than the 3rd larval instar	[48]
European earwig, *Furficula auricularia*	Aphid, Scale insects	*S. scapterisci*	Lab	Not infected	[55]
Carabid beetle *Bembidion properans, Pterostichus cupreus*	Pea weevil *Sitona lineatus*	*S.carpocapsae*	Field	No effect	[56]
Harmonia axyridis	Aphid, Scales, psyllids	*S.carpocapsae*	Lab	Causing temporary paralysis and death	[57]
Ladybugs *Coccinella undecimpunctat a*	Aphid	*H.taysearae S.carpocapsae* S2	Lab	High mortality of the larvae of the predator	[54]
Aphidoletis aphidimyza	Aphid	*S. carpocapsae, S.feltiae, H.bacteriophora*	Lab	9-93% infection	[49]
Coleomegilla maculata , *Olla v-nigrum, Harmonia axyridis , Coccinella septempunctata*	Aphid	*H.bacteriophora S.carpocapsae*	Lab	Less impact on lady beetle populations	[58]
Atheta coriaria	Fungus gnats	*S. feltiae*	Lab	No impact	[59]
Twospotted lady beetle, *Adalia*	Aphids	*S. feltiae, S. carpocapsae, H.bacteriophora*	Lab	Up to 100% mortality	[60];[50]

bipunctata, Lacewing *Chrysoperla carnea*					
European earwig, *Furficula auricularia*	Aphid, scale insects	*S. carpocapsae*	Lab	Highly infected(84.3% Mortality)	[51]
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	[61]
Dalotia coriaria	Western flower thrips, *Frankliniella occidentalis*, Fungus gnats *Bradysia* spp.	*H. bacteriophora* *S. feltiae* *S. carpocapsae* *S.riobrave*	Lab	Third instars were more susceptible than the adults	[62]
Macrolophus pygmaeus , *Nesidiocoris tenuis*	*Tuta absoluta*	*S. carpocapsae*	Pot	Not infected	[53]
Carabid beetle *Calosoma granulatum*	*Spodoptera frugiperda*	*H. amazonensis* RSC 5 , JPM 4	Lab	Safe	[63]
Green lacewing, *Chrysoperla carnea* Seven spotted lady beetle, *Coccinella septempunctata*	Pirate 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* , *S. glasseri* , *H. bacteriophora* HP88	Lab	Should avoid using concentrations above 100 IJs/ml of entomopathogenic nematodes during the peak of *C. carnea* and *C. septempunctata*	[64]

		H. marilatus MAR)			
Coccinella septumpunctata Chrysoperla carnea	*Spodoptera littoralis*	*H.bacteriophora*, *S. feltiae* , *S. carpocapsae*	Lab, Semi field	Low mortality	[65]
Coccinella undecimpunctata	Tortoise Beetle, *Cassida vittata*	*H.bacteriophora* H88 *S.carpocapsae* S2	Field	Safe	[66]

Most of the parasitoid larvae are not infected by EPNs within the host, but parasitoid larvae developing within infected hosts may be infected as they emerge from the host (Table2). Parasitoids cannot complete their development 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 distinguish between healthy and infected hosts. Kaya [67] observed that older endoparasitoids were less affected than young parasitoids by EPNs. 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 [68]. Once braconid larvae began cocoon spinning, there was no mortality. Wasps in fully formed cocoons were virtually immune to infection [69] as nematodes cannot penetrate. Though parasitoid larvae die when hosts are infected 2-3 d 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.

Table 2: Effect of entomopathogenic nematodes on nontarget parasitoid of insect pests

Parasitoids of insect pests	Insect pests	Entomopathogenic nematodes	Lab/ Field test	Effect on parasitoid	Reference
Braconid larval parasitoid, *Apanteles militaris*	Armyworm, *Pseudaletia unipuncta*	*Neoaplectana carpocapsae*, *Heterorhabditis heliothidis*	Lab	Deleterious effects on larvae, but not cocoon	[67]; [70]
Ichneumonid ,braconid	Tomato hornworms, Cabbageworms	*N.carpocapsae*	Lab	Deleterious effects	[71]
Olesicampe monticola	Larch sawfly *Cephalcia lariciphila*	*N. carpocapsae*	Lab	Deleterious effects	[72]
Tachinid parasitoid, *Myxecoristops* sp.	Sawfly, *Cephaleia abietis*	*Steinernema kraussei*	Lab	Deleterious effects	[52]
Tachinid parasitoid, *Compsilura*	Armyworm	*N. carpocapsae*	Lab	Nematodes were unable to develop within	[68]

concinnata				tachinid-parasitized hosts after the third day of parasitism by the parasitic insect	
Ichneumonid, *Xenoschesis fulvipes*, *Ctenopelma lucifer*	Spruce web-spinning sawfly, *Cephalcia arvensis*	*S.feltiae*	Field	66% reduction in emergence of *X. fulvipes*	[73]
Tachinid, *Ormia deplete*	Mole cricket, *Scapteriscus vicinus*	*S. scapterisci*	Lab	Not effected	[74]
Trichogramma chilonis, *T. japonicum*	*Corcyra cephalonica*	*H. indica*	Lab	Did not affect percent emergence	[75]
Bracon hylobii	*Hylobius abietis*	*S. carpocapsae* *H. downesi*	Field	Did not affect the natural populations	[76]
Cardiochiles diaphaniae	Melonworm *Diaphania hyalinata* , Pickleworm *D. nitidalis*	*S.carpocapsae*	Lab	Nematodes do not kill all parasitoids, the pupal stage is resistant to infection	[69]
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 the chance of wasp survival to adulthood.	[77]
Bracon hylobii	Large Pine Weevil,	*H.downesi*	Lab	Reduction in cocoon	[78]

	Hylobius abietis			formation,emerging adults are killed	
Trichogramma chilonis , *T. japonicum*	*Corcyra cephalonica*	*H.bacteriophora*	Lab	Did not affect percent emergence	[61]
Microplitis rufiventris	Cotton Leafworm, *Spodoptera littoralis*	*H.bacteriophora*, *S.carpocapsae*	Lab	Safe	[79]
Braconid , *Diachusmimorpha longicaudata*	Caribbean fruit fly, *Anastrepha suspensa*	*H. bacteriophora*	Field	Did not affect on natural population	[80]

Conclusion

With the increasing interest in use of EPNs as a biocontrol agent for pest control, the potential effects on non-targets beneficial insects are important and should be seriously taken into consideration. Hajek and Goettel, [81] stated that evaluation of entomopathogenic nematodes and other entomopathogens effects on nontarget organisms is an important yet relatively neglected area of study. A degree of caution may be advisable when purposely 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. When predators or parasitoids are present on the plants in high number, entomopathogenic nematodes should not be recommended for use. Newly developed EPN products must be evaluated for biosafety and environmental impact as well as delivery system.

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