Entomopathogenic Nematodes: Impacts on Non-target Invertebrates

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The sole author designed, analyzed, interpreted and prepared the manuscript.

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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.

Keywords: Bio-control agent; Entomopathogenic nematodes; predators; parasite and parasitoid; insect-pests.

1. 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].

1.1 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 & Loewe [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 trapezoides), 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, Neaopectana 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]; Zlotowska 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
Table 1. Effect of entomopathogenic nematodes on nontarget predator of insect pests

<table>
<thead>
<tr>
<th>Predators of insect pests</th>
<th>Insect pests</th>
<th>Entomopathogenic nematodes</th>
<th>Lab/Field test</th>
<th>Effect on predators</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thereva handlirschi, T. valida,</td>
<td>Sawfly, Cephalcia abietis</td>
<td>Steinernema kraussei</td>
<td>Lab</td>
<td>Did not infect the larvae of the predators</td>
<td>[52]</td>
</tr>
<tr>
<td>Rhagio notatus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earwig, Labidura riparia</td>
<td>Spodoptera littoralis</td>
<td>Heterorhabditis bacteriophora S. carpocapsae</td>
<td>Lab, Field</td>
<td>Only immature stages infected, Adult stages not infected</td>
<td>[26]</td>
</tr>
<tr>
<td>Philadelphia sp.</td>
<td>Fly maggot, mite</td>
<td>H. bacteriophora S. carpocapsae</td>
<td>Lab</td>
<td>Adult was less susceptible than the 3rd larval instar</td>
<td>[48]</td>
</tr>
<tr>
<td>European earwig, Furficula auricularia</td>
<td>Aphid, Scale insects</td>
<td>S. scapteriscia</td>
<td>Lab</td>
<td>Not infected</td>
<td>[55]</td>
</tr>
<tr>
<td>Carabid beetle</td>
<td>Pea weevil, Sitona lineatus</td>
<td>S. carpocapsae</td>
<td>Field</td>
<td>No effect</td>
<td>[56]</td>
</tr>
<tr>
<td>Harmonia axyridis</td>
<td>Aphid, Scales, psyllids</td>
<td>S. carpocapsae</td>
<td>Lab</td>
<td>Causing temporary paralysis and death</td>
<td>[57]</td>
</tr>
<tr>
<td>Ladybugs Coccinella undescephicpunctata</td>
<td>Aphid</td>
<td>H. taysearae S. carpocapsae S2</td>
<td>Lab</td>
<td>High mortality of the larvae of the predator</td>
<td>[54]</td>
</tr>
<tr>
<td>Aphidoletis aphidimyza</td>
<td>Aphid</td>
<td>S. carpocapsae, S. feltiae, H. bacteriophora</td>
<td>Lab</td>
<td>9-93% infection</td>
<td>[49]</td>
</tr>
<tr>
<td>Coleomegilla maculata, Olla v-nigrum,</td>
<td>Aphid</td>
<td>H. bacteriophora S. carpocapsae</td>
<td>Lab</td>
<td>Less impact on lady beetle populations</td>
<td>[58]</td>
</tr>
<tr>
<td>Harmonia axyridis</td>
<td>Aphid</td>
<td>S. carpocapsae</td>
<td>Lab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coccinella septempunctata</td>
<td>Aphid</td>
<td>S. feltiae</td>
<td>Lab</td>
<td>No impact</td>
<td>[59]</td>
</tr>
<tr>
<td>Atheta coriaria</td>
<td>Fungus gnats</td>
<td>S. feltiae</td>
<td>Lab</td>
<td>Up to 100% mortality</td>
<td>[60];[50]</td>
</tr>
<tr>
<td>Twospotted lady beetle, Adalia bipunctata,</td>
<td>Aphid</td>
<td>S. feltiae, S. carpocapsae, H. bacteriophora</td>
<td>Lab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lacewing Chrysoperla carnea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European earwig, Furficula auricularia</td>
<td>Aphid, Scale insects</td>
<td>S. carpocapsae</td>
<td>Lab</td>
<td>Highly infected (84.3% Mortality)</td>
<td>[51]</td>
</tr>
<tr>
<td>Predators of insect pests</td>
<td>Insect pests</td>
<td>Entomopathogenic nematodes</td>
<td>Lab/Field test</td>
<td>Effect on predators</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------</td>
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</tr>
<tr>
<td>Chrysoperla zastrowi</td>
<td>Mealybugs, Aphids, Thrips, Psyllids, Whiteflies</td>
<td>H. bacteriophora</td>
<td>Lab</td>
<td>Nematode did not affect egg-hatching and survival of larvae or adults of the predator</td>
<td>[61]</td>
</tr>
<tr>
<td>Dalotia coriaria</td>
<td>Western flower thrips, Frankliniella occidentalis, Fungus gnats Bradysia spp.</td>
<td>H. bacteriophora S. feltiae S. carpocapsae S. riobrave</td>
<td>Lab</td>
<td>Third instars were more susceptible than the adults</td>
<td>[62]</td>
</tr>
<tr>
<td>Macrolaphus pygmaeus , Nesidiocoris tenuis</td>
<td>Tuta absoluta</td>
<td>S. carpocapsae</td>
<td>Pot</td>
<td>Not infected</td>
<td>[53]</td>
</tr>
<tr>
<td>Carabid beetle Calosoma granulatum</td>
<td>Spodoptera frugiperda</td>
<td>H. amazonensis RSC 5 , JPM 4</td>
<td>Lab</td>
<td>Safe</td>
<td>[63]</td>
</tr>
<tr>
<td>Green lacewing, Chrysoperla carnea Seven spotted lady beetle, Coccinella septempunctata</td>
<td>Pirate bug, Orius albidipennis</td>
<td>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. glasser, H. bacteriophora HP88, H. marilatus MAR)</td>
<td>Lab</td>
<td>Should avoid using concentrations above 100 IJs/ml of entomopathogenic nematodes during the peak of C. carnea and C. septempunctata</td>
<td>[64]</td>
</tr>
<tr>
<td>Coccinella septempunctata Chrysoperla carnea</td>
<td>Spodoptera littoralis</td>
<td>H. bacteriophora, S. feltiae, S. carpocapsae</td>
<td>Lab, Semi field</td>
<td>Low mortality</td>
<td>[65]</td>
</tr>
<tr>
<td>Coccinella undecimpunctata</td>
<td>Tortoise Beetle, Cassida vittata</td>
<td>H.bacteriophora H88, S.carpocapsae S2</td>
<td>Field</td>
<td>Safe</td>
<td>[66]</td>
</tr>
</tbody>
</table>
Table 2. Effect of entomopathogenic nematodes on nontarget parasitoid of insect pests

<table>
<thead>
<tr>
<th>Parasitoids of insect pests</th>
<th>Insect pests</th>
<th>Entomopathogenic nematodes</th>
<th>Lab/Field test</th>
<th>Effect on parasitoid</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braconid larval parasitoid, Apanteles militaris</td>
<td>Armyworm, Pseudaletia unipuncta</td>
<td>Neoaplectana carpopcapsae, Heterorhabditis heliothidis</td>
<td>Lab</td>
<td>Deleterious effects on larvae, but not cocoon</td>
<td>[67]; [70]</td>
</tr>
<tr>
<td>Ichneumonid, braconid</td>
<td>Tomato hornworms, Cabbage worms</td>
<td>N. carpocapsae</td>
<td>Lab</td>
<td>Deleterious effects</td>
<td>[71]</td>
</tr>
<tr>
<td>Olesicampe monticola</td>
<td>Larch sawfly Cephalcia lariciphila</td>
<td>N. carpocapsae</td>
<td>Lab</td>
<td>Deleterious effects</td>
<td>[72]</td>
</tr>
<tr>
<td>Tachinid parasitoid, Myxecoristops sp.</td>
<td>Sawfly, Cephelea abietis</td>
<td>Steinernema kraussei</td>
<td>Lab</td>
<td>Deleterious effects</td>
<td>[52]</td>
</tr>
<tr>
<td>Tachinid parasitoid, Compsilura concinnata</td>
<td>Armyworm</td>
<td>N. carpocapsae</td>
<td>Lab</td>
<td>Nematodes were unable to develop within tachinid-parasitized hosts after the third day of parasitism by the parasitic insect</td>
<td>[68]</td>
</tr>
<tr>
<td>Ichneumonid, Xenoschesis fulvipes, Clenopelma lucifer</td>
<td>Spruce web-spinning sawfly, Cephalcia arvensis</td>
<td>S. feltiae</td>
<td>Field</td>
<td>66% reduction in emergence of X. fulvipes</td>
<td>[73]</td>
</tr>
<tr>
<td>Tachinid, Ormia deplete</td>
<td>Mole cricket, Scapteriscus vicinus</td>
<td>S. scapterisci</td>
<td>Lab</td>
<td>Not effected</td>
<td>[74]</td>
</tr>
<tr>
<td>Trichogramma chilonis, T. japonicum</td>
<td>Corcyra cephalonica</td>
<td>H. indica</td>
<td>Lab</td>
<td>Did not affect percent emergence</td>
<td>[75]</td>
</tr>
<tr>
<td>Bracon hylobii</td>
<td>Hylobius abietis</td>
<td>S. carpocapsae H. downesi</td>
<td>Field</td>
<td>Did not affect the natural populations</td>
<td>[76]</td>
</tr>
<tr>
<td>Cardiochiles diaphaniae</td>
<td>Melonworm Diaphania hyalinata, Pickleworm D. nitidalis</td>
<td>S. carpocapsae</td>
<td>Lab</td>
<td>Nematodes do not kill all parasitoids, the pupal stage is resistant to infection</td>
<td>[69]</td>
</tr>
<tr>
<td>Eulophid parasitoid wasp Diglyphus begini</td>
<td>Leafminer Liriomyza trifolii</td>
<td>S. carpocapsae</td>
<td>Lab</td>
<td>Adult D. begini not susceptible to nematode infection, but avoid ovipositing on</td>
<td>[77]</td>
</tr>
</tbody>
</table>
Parasitoids of insect pests | Insect pests | Entomopathogenic nematodes | Lab/Field test | Effect on parasitoid | Reference |
---|---|---|---|---|---|
| Bracon hylobii | Large Pine Weevil, *Hylobius abietis* | *H. downesi* | Lab | Reduction in cocoon formation, emerging adults are killed | [78] |
| 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, *Diachus mimorpha longicaudata* | Caribbean fruit fly, *Anastrepha suspensa* | *H. bacteriophora* | Field | Did not affect on natural population | [80] |

nematode-infected larvae. However, the presence of nematodes in mines with wasp eggs decreased the chance of wasp survival to adulthood.
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.

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 (Table 2). 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 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.

2. 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.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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