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Effect of Entomopathogenic Nematodes (Nematoda: Rhabitida) on Earthworms, Spiders and Ants

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Abstract

Steinernematids and Heterorhabditids are lethal insect pathogens with bio control potential for managing insect pests of agricultural importance. The non-target effects of entomopathogenic nematodes (EPNs) Steinernema thermophilum, S. glaseri and H. indica were evaluated on beneficial organisms like earthworm (Eiseinea foetida), Spider (Neoscona theisi) and ant (Messor himalayanus). These nematodes were found to be safe towards the earthworm species E. foetida as it did not cause any earthworm mortality up to 3 weeks of treatment when applied @ 500 Infective Juveniles (IJ)/gram compost. However, S. thermophilum, S. glaseri and H. indica induced mortality of 20, 10 and 30 %, respectively on Spider species Neoscona theisi when applied @ 500 IJs/spider, but no mortality was recorded @ 100 IJs/spider. The S. thermophilum and H. indica when applied on ant species M. himalayanus @ 100 IJs/ant could induce 100 % mortality, whereas S. glaseri caused 72 % ant mortality after 5 days. Our study revealed the safety of EPNs towards earthworms but possible adverse effects on spiders and ants.

Keywords: *Steinernema*, *Heterorhabditis*, Non target effect.

Introduction

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are naturally occurring pathogens of insects[1 2] which are environmental friendly alternative for insect pest management[1 3]. Most bio control agents require days or weeks to kill their hosts, yet nematodes, working with their symbiotic bacteria, kill insects within 24 to 48 hours. The non-feeding infective juvenile seeks out insect hosts, and penetrates into the insect body, usually through natural body openings (mouth, anus, spiracles) or areas of thin cuticle. These nematodes carry symbiotic bacteria (*Xenorhabdus* for steinernematids and *Photorhabdus* for herterorhabditids), which are released in the insect haemocoel where the bacteria multiply and cause septicemia, thus resulting in the mortality of the insect host. The nematodes feed upon the bacteria, liquefies insect and mature into adults. Thus, entomopathogenic nematodes are a nematode-bacterium complex. They are safe for the plant health, human health, soil and the environment and hence are exempted from registration in many developed countries. However, studies on the non target effects of entomopathogenic nematodes on various species of non target organisms showed a large range from complete harmlessness to pronounced harmful effect[4-6]. The results of some field trials showed a moderate influence of these nematodes on non target arthropods or even the absence of such an effect[7]. Bathon[4] reported that mortality can be observed among the non target organisms, but the influence of these agents should be temporary and local and so only a part of the population is under attack. Georgis *et al*.[7] demonstrated a negligible influence of entomopathogenic nematodes on non target organisms when used only in short-term pest control. Earthworms are one of the most important beneficial organism in the agricultural field as it improve soil conditions (aeration, drainage and organic matter content) and they are able to change soil structure, move large amounts of soil and affect micro floral and faunal diversity[8 9]. Besides, many associations (phoretic, paratenic intermediate or sole host) between nematodes and earthworms had been reported[10-12], and some authors think that earthworms could be used as vectors to introduce and/or disperse beneficial organisms[13-15]. Most of the spiders are predators which feed on insect pests of agricultural importance. Most ant species present in agricultural field perform many ecological roles that are beneficial to humans, including the suppression of pest populations and aeration of the soil. Hence, the present study was carried out to evaluate the non target effect of indigenous entomopathogenic nematodes *Steinernema thermophilum*[16], *S. glaseri*[17],[18], and *Heterorhabditis indica*[19] against epigeic earthworm (*Eisenia fetida*), spider (*Neoscona theisi*) and ant species (*Messor himalayanus*).

Material and Methods

Nematodes: The non target effect of different entomopathogenic nematodes (EPNs) namely *Steinernema thermophilum*, *S. glaseri* and *Heterorhabditis indica* were evaluated. The pure cultures of Infective juveniles (IJs) of these EPNs already available in our laboratory were maintained on 4th instar larvae of greater wax moth, *Galleria mellonella* (L.)[20]. The emerging IJs from the insect cadavers were harvested in sterile distilled water using a modified White's trap, and stored at 15^o C in BOD incubator. The IJs for the study were used within 2-4 weeks of storage.

Beneficial organisms used: The impact of entomopathogenic nematodes, *S. thermophilum, S. glaseri and H. indica* was evaluated on ant (*Messor himalayanus*), spider (*Neoscona theisi;* Family: Araenidae) and earthworm (*Eisinea foetida*). The earthworm species was procured from the Division of Entomology, IARI, New Delhi, whereas the spiders and ants were collected directly from IARI Research Farm.

Effect of entomopathogenic nematodes on ant: The experiment was carried out in transparent plastic plates of 10 mm diameter lined with 2 layers of Whatman No. 1 filter paper. Each plate was provided with 50 µl of honey solution in small containers to feed the ants. The infective juveniles of entomopathogenic nematodes were released @ 1000 IJs/plate in 300 µl of sterile distilled water. Ten ants were released in each plate to yield a dosage of 100 IJs/ant. All the treatments were replicated five times and the experiment was held at 25$\pm$1 ^{o}C. The observations on mortality of ants were taken at every 24 h interval.

Effect of entomopathogenic nematodes on spider: The experiment was carried out in 12-well plates lined with two layers of Whatman No. 1 filter paper. Two doses (100 IJs/well and 500 IJs/well) of the three species of entomopathogenic nematodes in 100 µl of sterile distilled water were applied in the each well of twelve well plates. After the release of IJs in the wells, the spiders were released as one spider/well of the 12-well plates. Ten spiders were included in each treatment and experiment was held at 25$\pm$1 ^{o}C. The observations on mortality of spiders were recorded in 24 h intervals. The cadavers were observed for the emergence of nematode progenies.

Effect of entomopathogenic nematodes on earthworms: The experiment was carried out in 100 ml capacity plastic bottles containing 20 g of vermicompost. The EPN species were added @ 100 IJs/g compost and 500 IJs/g compost in each bottles and the earthworms were released @ 4 earthworms/bottle. All the treatments were replicated five times and held at 25$\pm$1 ^{o}C. The observations on survival of earthworms were recorded on 7th, 14th and 21st day of the treatment. Fourth instar larvae of *G. mellenella* @ 4 larvae/bottle were added on 10th day of treatment to confirm the presence and survival of IJs in the vermicompost. The dead larvae from each bottle were collected in 24 hour intervals.

Results and Discussion

Entomopathogenic nematodes, *S. thermophilum*, *S. glaseri* and *H. indica* were treated @ 100 IJs/ant on the adults of ant species *Messor himalayanus*. Among these three species, *S. thermophilum* observed to be more pathogenic followed by *H. indica* and least by *S. glaseri,* in all the time durations evaluated. The data on mortality are shown in table-1. A mortality level of 68 % after 24 hours and 100 % in 120 hours were induced by *Steinernema thermophilum*. Even though, *H. indica* induced only 40 % mortality in 24 h, it yielded 100 % in 120 h. However, *S. glaseri* was found to be least pathogenic against *M. himalayanus* as it induced only 24 % mortality after 24 h and a maximum of only 72 % in 120 h of treatment application. The levels of mortality induced by *S. glaseri* were found to be statistically significant as compared with the mortality induced by *S. thermophilum* in all the four time intervals evaluated. The data on mortality caused by *S. glaseri* after 120 h of treatment indicated significant difference from the treatments of both *S. thermophilum* and *H. indica* in the same time duration. Our study revealed, the possible threat of entomopathogenic nematodes to ant species inhabiting agricultural field, given the fact that the soil is the natural habitat of both EPNs and ants.

Table-1
Mortality of ant species, *Messor himalayanus* by 3 entomopathogenic nematode species when applied at the rate of 100 Infective juveniles per ant

Treatments	% Mortality				
	24 Hrs.	**48 Hrs.**	**72 Hrs.**	**96 Hrs.**	**120 Hrs.**
S. thermophilum	68.0 (55.6)	86.0 (68.1)	92.0 (73.6)	98.0 (81.9)	100.0 (90.0)
S. glaseri	24. (29.3)	32.0 (34.5)	40.0 (39.3)	46.0 (42.7)	72.0 (58.1)
H. indica	40.0 (39.3)	48.0 (43.9)	64.0 (53.2)	78.0 (62.1)	100.0 (90.0)
Control	0.0 (0.4)	0.0 (0.4)	0.0 (0.4)	0.0 (0.4)	0.0 (0.4)
SEM	6.1	4.5	5.4	3.9	2.8
CD (P= 0.05)	17.0	12.5	15.1	10.8	7.8

Adult stages of the spider, *Neoscona theisi* were treated with entomopathogenic nematodes at two different doses. Maximum of only 30, 20 and 10 % mortality was recorded by *H. indica, S. thermophilum* and *S, glaseri* respectively when applied @ 500 IJs/spider in 120 h after treatment. But there was no record of mortality of spiders up to 120 h in any of the treatments when these EPNs applied @ 50 IJs/spider. Hence, all the three species of EPNs evaluated were observed to be potentially pathogenic to spiders at higher doses, with least effect by *S. glaseri*. The data on the mortality of spiders in different treatments are given in table-2. The emergence of infective juveniles from the cadavers of spider confirmed the infection of entomopathogenic nematodes. Earlier Bathon[4] reported the pathogenicity of entomopathogenic nematode, *H. bacteriophora* on spiders under laboratory conditions. But he could not observe any effect under natural conditions. This could be due to prohibition of natural habitat of these organisms from nematode infection. In the present study, we could observe only a maximum of 30 % spider mortality even at a higher dose of 500 IJs/spider, a dose which is unlikely to encounter in the natural habitat of spiders. So there is more possibility of safety of EPNs against spiders under field conditions. But it indicated the need for precautions before the field application of EPNs, as we observed the mortality of spiders, even though at a lower level in very high dose. It would be better to go for field application at a time when spiders are least active.

Table-2
Mortality of Spider species, *Neoscona theisi* by two different doses of infective juveniles (IJs) of 3 entomopathogenic nematode species

Treatment	% Mortality				
	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
S. thermophilum @ 50 IJs	00	00	00	00	00
S. thermophilum @ 500 IJs	10	20	20	20	20
S. glaseri @ 50 IJs	00	00	00	00	00
S. glaseri @ 500 IJs	00	10	10	10	10
H. indica @ 50 IJs	00	00	00	00	00
H. indica @ 500 IJs	00	20	30	30	30

The entomopathogenic nematodes, *S. thermophilum*, *S. glaseri* and *H. indica* were applied on the earthworms, *Eisinia foetida* in the vermicompost medium @ 100 IJs/g and 500 IJs/g compost and observed for the mortality/survival of earthworms up to 21 days. There was no record of mortality of earthworms in any of the treatments evaluated. The survival of infective juveniles of these nematodes was evaluated by putting 4th instar larvae of *G. mellonella* in each treatments after 10 days of starting the experiment. As 100 % of larval mortality was recorded in all the treatments within 48 h of exposure, this study confirmed the survival of infective juveniles of these EPNs in the vermicompost medium without causing any harmful effect on the earthworms. The data on the study is presented in Table-3. Earlier several authors have observed the non-susceptibility of earthworms to different steinernematids[14,21,22] and to the slug-parasitic nematode *Phasmorhabditis hermafrodita* Schneider (Nematoda: Rhabditidae)[23]. Some authors think that earthworms could be used as vectors to introduce and/or disperse beneficial organisms including EPNs[13-15]. Raquel *et al.*[24] studied the interaction of *S. feltiae* with earthworm species *Eisenia fetida* and reported about 20 – 90 % of the earthworms as transmitting nematodes through their gut without any pathogenic effect on earthworms. However they observed a reduction in the mobility of infective juveniles of *S. feltiae* after passage through the *E. fetida* gut. The present study concluded the safety of EPN species studied towards earthworms as we could not observe any harmful effect of earthworms exposed to very high dose of EPNs for a period of 3 weeks.

Table-3
Mortality/survival of earthworm species, *Eisenia foetida* and fourth instar larvae of *Galleria mellonella* (Greater wax moth) by infective juveniles (IJs) of 3 entomopathogenic nematodes species

Treatment	% Mortality					
	Earthworms			Greater wax moth larvae		
	7 Days	14 Days	21 Days	24 Hrs	48 Hrs	72 Hrs
S. thermophilum @ 250 IJs/g	00	00	00	5	100	100
S. thermophilum @ 100 IJs/g	00	00	00	00	100	100
S. glaseri @ 250 IJs/g	00	00	00	00	100	100
S. glaseri @ 100 IJs/g	00	00	00	00	100	100
H. indica @ 250 IJs/g	00	00	00	00	100	100
H. indica @ 100 IJs/g	00	00	00	00	100	100
Control	00	00	00	00	00	00

Conclusion

Non target effect of entomopathogenic nematodes namely *S. thermophilum*, *S. glaseri* or *H. indica* were evaluated on ant, spider and earthworm. The study observed no pathogenic effect of these EPNs towards earthworm species, *E. foetida* even at very higher dose. So, we concluded the safety of earthworms by these nematodes. However these EPNs at higher doses were found to be lethal to spider species, *N. theisi* and ant species *M. himalayanus* at higher doses. Even though spiders could potentially escape the infection of EPNs, because of the habitat characteristics, it could be a potential threat to ants. So these EPN species should be applied at the time when beneficial ants or spiders are least active.

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