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Compatibility of different strains of *Steinernema* spp. and *Heterorhabditis* spp. against tobacco caterpillar, *Spodoptera litura*

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Abstract
The experiments were conducted under laboratory, Department of Nematology, CCS HAU, Hisar during the month of September-October, 2016-17. The virulence of five strains of *Steinernema abbasi* and two *Heterorhabditis indica* were evaluated against *Spodoptera litura* in four doses (5, 10, 20 and 40IJs/insect larvae) under in *vitro* conditions. As far as effect of different IJs is concerned, the significant mortality in larvae started at 5IJs/insect larvae However, nearly 50 per cent mortality of the larvae was observed at an inoculum level of 10IJs per larva after 24h. Among all the isolates of EPNs, *S. abbasi* isolate HAR-EPN-Sa-3 was highly virulent against *S. litura*. In case of *H. indica,* isolate HAR-EPN-Hi-2 was highly virulent and HAR-EPN-Hi-1 recorded the least virulence against *S. litura*. Laboratory studies revealed that *S. abbasi* in combination with *H. indica* had more virulence than *S. abbasi* and *H. indica* when applied alone. After 72 and 96h, both (H+S) caused 97.3 and 99.7 percent mortality. There was a mean death of 39.7 per cent and at 20IJs, 73.7 per cent mortality of *S. litura* could be recorded.

Keywords: Compatibility, entomopathogenic nematodes, *Heterorhabditis indica*, *Spodoptera litura, Steinernema abbasi*

Introduction
Entomopathogenic nematodes (EPNs) have a great potential as biological control agents of insect pests of crops due to their wide host range, ease to handle, short life cycle and environmental safety [13]. They have the ability to search for hosts and, due to their high reproductive potential, have the ability to react quickly to changing pest densities; they are non-toxic to humans and considered safe to the environment, and they can often be mass cultured, formulated and then applied like agricultural chemicals [8].
Steinernematid and Heterorhabditid nematodes carry symbiotic bacteria namely *Xenorhabdus* spp and *Photorhabdus* spp, respectively in their gut [2] which are released in the insect haemoceol wherein the bacteria multiply and cause septicemia thus killing the host within 24-48 hours [14][19]. The local strains of EPNs may be more effective as biocontrol agents against local insect pests because of their adaptability to climatic conditions of that area.
Tobacco caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is a polyphagous insect pest of national importance causing economic damage to a number of agricultural crops. It is known to damage more than 50 crops viz., tobacco, cole crops, castor, cotton, sunflower, chilli, etc. [11]. Several synthetic insecticides were tested and recommended to control this pest. However, indiscriminate and frequent application of these chemicals resulted in problems like buildup of resistance, secondary pest resurgence and environmental pollution. Native isolates of biological control agents are better adapted to the local agro-climatic conditions as compared to exotic species; therefore, the present study was carried out on the compatibility of different strains of two native EPNs *viz*., *Steinernema abbasi* and *Heterorhabditis indica* and their efficacy against tobacco caterpillar, *Spodoptera litura.*

Materials and Methods
The soil samples were taken from field area of CCS HAU, Hisar and other parts of Haryana for isolation of EPNs. The strains were isolated and tested against tobacco caterpillar, *Spodoptera litura*. The study was carried out in laboratory conditions of Department of Nematology, CCS HAU, Hisar during the month of September- October, 2016. Five strains of *S. abbasi* and two of *H. indica* were used in the experiments for their virulence on *Spodoptera*

litura, representing 10 different geographical regions of Haryana. The virulence of *S. abbasi* and *H. indica* to *S. litura* was determined by inoculating with nematode infective juveniles in different concentrations @ 5, 10, 20 and 40IJs in 200 µl distilled water per ten larvae with distilled water without nematodes treated as check. The treatments were arranged in a complete randomized design. The EPNs strains identified as virulent in experiment No. 1 were used for compatibility studies. For present studies, the strains at the pathogenic level i.e 10IJs/larvae were injected in 10 larvae of *S. litura***.**

Treatments were replicated four times at each concentration under room temperature (25 ± 1 °C). Observations on the mortality of larvae were recorded 1, 2, 3 and 4 days after inoculation.

Statistical analysis

The data obtained in the experiment was analysed statistically using sine arc transformation. The ratio of dead insect larvae/number of total insect larvae expressed the percentage mortality as follows:

Mortality (%) = D x 100 / N

Where:

D-Number of dead larvae

N-Total number of larvae

Results and Discussion

In the first study, all the tested seven strains of EPNs were pathogenic to *S. litura* larvae. Results revealed that both the strains of EPN were found to be virulent and could cause more than 50 per cent mortality at 10IJs/larva under laboratory conditions. Among all the isolates of EPNs, *S. abbasi* isolate HAR-EPN-Sa-3 was highly virulent against *S. litura*. It was followed by other isolates in the order of HAR-EPN-Sa-2, HAR-EPN-Sa-5 and HAR-EPN-Sa-4. HAR-EPN-Sa-1 recorded the least virulence of all the isolates (Table 1-5). As far as effect of different IJs is concerned, the significant mortality of larvae started at 5IJs/insect larva. However, nearly 50 per cent mortality of the larvae was observed at an inoculum level of 10IJs per larva after 24h. Similar, findings were also reported by Rajkumar [15] who observed that the mortality ranged between 16.7 to 88.9 per cent under different inoculum levels and time of exposure. It further increased with an increase in the inoculum levels and period of exposure.

Table 1: *In vitro* larval mortality of *S. abbasi* strain HAR-EPN-Sa-1 against *S. litura.*

Treatments (IJs/petri plate)	Larval mortality at different time intervals (h)				
	24	48	72	96	Mean
T_1 (Control)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2 (5 IJs)	7.6 (12.7)	20.0 (26.2)	37.5 (37.6)	52.5 (46.5)	29.4 (30.7)
T_3 (10 IJs)	40.0 (39.1)	60.0 (50.8)	75.0 (60.0)	89.9 (73.4)	66.2 (55.9)
T_4 (20 IJs)	67.5 (55.5)	80.0 (63.8)	89.9 (73.4)	97.3 (83.2)	83.7 (69.0)
T_5 (40 IJs)	87.4 (71.4)	94.9 (79.3)	97.3 (83.2)	99.7 (87.1)	94.8 (80.2)
Mean	50.7 (36.3)	63.7 (44.6)	74.9 (51.4)	84.9 (58.6)	

Values in parenthesis are angular transformed values.

C.D. at 5% Time:- (4.3), Dosages:- (4.9), Interaction (Time v/s Dosages):- (9.7)

Table 2: *In vitro* larval mortality of *S. abbasi* strain HAR-EPN-Sa-2 against *S. litura.*

Treatments (IJs/petri plate)	Larval mortality at different time intervals (h)				
	24	48	72	96	Mean
T_1 (Control)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2 (5 IJs)	10.1 (14.7)	26.0 (30.0)	40.0 (39.0)	55.0 (47.9)	32.8 (32.9)
T_3 (10 IJs)	47.5 (43.5)	67.5 (55.4)	82.5 (65.8)	89.9 (73.4)	71.9 (59.5)
T_4 (20 IJs)	65.0 (54.0)	77.5 (62.6)	87.4 (71.4)	94.9 (79.3)	81.2 (66.8)
T_5 (40 IJs)	80.0 (64.3)	92.4 (77.3)	97.3 (83.2)	99.7 (87.1)	92.4 (78.0)
Mean	50.6 (35.9)	65.8 (45.6)	76.8 (52.5)	84.9 (58.1)	

Values in parenthesis are angular transformed values.

C.D. at 5% Time:- (5.1), Dosages:- (5.7), Interaction (Time v/s Dosages):- (11.4)

Table 3: *In vitro* larval mortality of *S. abbasi* strain HAR-EPN-Sa-3 against *S. litura.*

Treatments (IJs/petri plate)	Larval mortality at different time intervals (h)				
	24	48	72	96	Mean
T_1 (Control)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2 (5 IJs)	17.5 (24.1)	35.0 (35.9)	62.5 (52.5)	84.9 (69.7)	50.0 (45.6)
T_3 (10 IJs)	50.0 (45.0)	75.0 (60.6)	87.4 (71.4)	92.4 (77.3)	76.2 (63.6)
T_4 (20 IJs)	75.0 (60.6)	89.9 (73.4)	92.4 (77.3)	97.3 (83.2)	88.6 (73.6)
T_5 (40 IJs)	84.9 (69.7)	94.9 (79.3)	99.7 (87.1)	99.7 (87.1)	94.9 (80.8)
Mean	56.9 (40.4)	73.7 (50.4)	85.5 (58.2)	93.6 (64.0)	

Values in parenthesis are angular transformed values.

C.D. at 5% Time: - (5.4), Dosages:- (6.0), Interaction (Time v/s Dosages):- (12.0)

Table 4: *In vitro* larval mortality of *S. abbasi* strain HAR-EPN-Sa-4 against *S. litura.*

Treatments (IJs/petri plate)	Larval mortality at different time intervals (h)				
	24	48	72	96	Mean
T_1 (Control)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2 (5 IJs)	17.5 (24.1)	30.0 (33.0)	50.0 (45.0)	70.0 (56.9)	41.9 (39.8)
T_3 (10 IJs)	42.5 (40.4)	57.5 (49.4)	75.0 (60.6)	89.9 (73.4)	66.2 (55.9)
T_4 (20 IJs)	60.0 (51.0)	72.5 (59.1)	87.4 (71.4)	94.9 (79.3)	78.7 (65.2)
T_5 (40 IJs)	70.0 (56.9)	82.5 (65.4)	94.8 (79.3)	99.7 (87.1)	86.8 (72.2)
Mean	47.5 (35.1)	60.6 (42.0)	76.8 (51.8)	88.6 (59.9)	

Values in parenthesis are angular transformed values.

C.D. at 5% Time:- (4.5), Dosages:- (5.1), Interaction (Time v/s Dosages):- (10.1)

Table 5: *In vitro* larval mortality of *S. abbasi* strain HAR-EPN-Sa-5 against *S. litura.*

Treatments (IJs/petri plate)	Larval mortality at different time intervals (h)				
	24	48	72	96	Mean
T_1 (Control)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2 (5 IJs)	10.1 (16.6)	27.5 (31.4)	50.0 (45.0)	67.5 (55.6)	38.8 (37.1)
T_3 (10 IJs)	42.5 (40.6)	60.0 (51.0)	79.9 (66.0)	84.4 (71.4)	66.7 (57.2)
T_4 (20 IJs)	55.0 (47.9)	72.5 (58.6)	87.4 (71.4)	94.9 (79.3)	77.4 (64.3)
T_5 (40 IJs)	72.5 (58.6)	84.9 (69.7)	94.9 (79.3)	97.3 (83.2)	87.4 (72.7)
Mean	45.0 (33.3)	61.2 (42.7)	79.1 (52.9)	86.0 (58.5)	

Values in parenthesis are angular transformed values.
C.D. at 5% Time:- (5.9), Dosages:- (6.0), Interaction (Time v/s Dosages):- (12.1)

The data on the relative mortality by *S. abbasi* to *S. litura* indicated that the lowest level of 5IJs required about 45.4h or approximately two days for causing death of 50 per cent larvae and the maximum level of 40IJs required only 1.1h (Table 6). To cause 50 per cent mortality of the host insect within 24h, it required about 9.1IJs. Similarly, a dosage of 5.6 for 48h, followed by 1.7 and 0.7IJs resulted in 72 and 96h respectively (Table 7). Tohirand [17] also reported that there was linear relationship between dosage of nematodes and larval morality. In the present studies, high larval mortality of *S. litura* was observed at high inoculum level of 40IJs/larvae. The HAR-EPN-Sa-3 caused 50 percent mortality of *S. litura* in a minimum time which was followed by HAR-EPN-Sa-2. Maximum time was taken by HAR-EPN-Sa-1.

Table 6: Time mortality response of *S. litura* to different dosages of *S. abbasi* (LT 50).

(IJs/larva)	LT 50 (h)	Regression equation
5	45.4	y = 4.78 ln(x)-14.00
10	20.4	y = 3.14 ln(x)-4.84
20	4.2	y = 1.58 ln(x)+2.60
40	1.05	y = 1.15 ln(x) +4.95

Table 7: Susceptibility of *S. litura* to *S.abbasi* at different time interval (LD 50).

Time interval (h)	LD 50 (IJs/larva)	Regression equation
24	9.13	y = 3.28 ln(x) – 3.01
48	5.56	y = 2.81 ln(x) – 0.08
72	1.69	y = 1.69 ln(x) +4.07
96	0.72	y = 0.72 ln(x) +0.03

In case of *H. indica* isolate, HAR-EPN-Hi-2 was highly virulent and HAR-EPN-Hi-1 recorded the least virulence against *S. litura* (Table 8-9). However, on an average, significant effect of time interval was observed irrespective of the nematode inoculum levels. At maximum dose of 40IJs per larvae there was an average 80.6 per cent mortality of the insect.

Table 8: *In vitro* larval mortality of *S. abbasi* strain HAR-EPN-Hi-1 against *S. litura.*

Treatments (IJs/petri plate)	Larval mortality at different time intervals (h)				
	24	48	72	96	Mean
T_1 (Control)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2 (5 IJs)	12.5 (20.5)	25.0 (29.9)	50.0 (45.0)	70.0 (57.0)	39.7 (38.1)
T_3 (10 IJs)	32.5 (34.5)	50.0 (45.0)	72.50 (58.6)	89.9 (73.4)	61.2 (52.9)
T_4 (20 IJs)	50.0 (45.0)	65.0 (53.8)	85.0 (67.5)	94.9 (79.3)	73.7 (61.4)
T_5 (40 IJs)	60.0 (50.8)	77.5 (62.1)	87.4 (71.4)	97.3 (83.2)	80.6 (66.9)
Mean	38.7 (30.7)	54.4 (38.7)	73.7 (49.0)	88.0 (59.1)	

Values in parenthesis are angular transformed values.
C.D. at 5% Time:- (3.7), Dosages:- (4.1), Interaction (Time v/s Dosages):- (8.2)

Table 9: *In vitro* larval mortality of *S. abbasi* strain HAR-EPN-Hi-2 against *S. litura.*

Treatments (IJs/petri plate)		Larval mortality at different time intervals (h)			
	24	48	72	96	Mean
T_1 (Control)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2 (5 IJs)	7.6 (12.7)	25.0 (29.3)	42.5 (40.6)	60.0 (50.8)	33.8 (33.4)
T_3 (10 IJs)	47.5 (43.5)	62.5 (52.3)	80.0 (63.8)	89.9 (73.4)	70.0 (58.2)
T_4 (20 IJs)	57.5 (49.4)	70.0 (57.1)	84.9 (69.7)	92.4 (77.3)	76.2 (63.4)
T_5 (40 IJs)	72.5 (58.6)	82.5 (65.4)	89.9 (73.4)	97.3 (83.2)	85.6 (70.2)
Mean	46.3 (33.4)	60.0 (41.4)	74.3 (50.1)	84.9 (57.5)	

Values in parenthesis are angular transformed values.
C.D. at 5% Time:- (4.8), Dosages:- (5.4), Interaction (Time v/s Dosages):- (10.8)

However, minimum dosage of 10IJs per larva resulted in 50.0 per cent mortality immediately after 48h. Umamaheshwari [18] recorded 75.6 per cent mortality of *S. litura* larvae after 72h of treatment with *H. indica*. The reproductive potential of nematode was not affected by the level of IJs [5]. As the level of infective juveniles increased, there was a significant decrease in time required for causing 50 per cent mortality of the insect. The results on median lethal dose of *H. indica* required for 50 per cent mortality of *S. litura* revealed that the LD50 value was 10.1IJs per larva recorded after 24h. If the mortality has to occur within 96h, an individual larva required about 1.6IJs (Table 11). Further, it was clear that LD50 and LT50 value for both nematodes were decreased with increase in time and dosage, respectively (Table 10). Parihar [12] observed 50 per cent larval mortality within 72h of exposure at an inoculum level of 100IJs of *Heterorhabditis*.

Table 10: Time mortality response of *S. litura* to different dosages of *H.indica* (LT 50).

(IJs/larva)	LT 50 (h)	Regression equation
5	48.0	y = 3.68 ln(x)-11.26
10	23.7	y = 3.09 ln(x)-5.28
20	16.5	y = 2.56 ln(x)+2.56
40	6.5	y = 1.76 ln(x)+1.56

Table 11: Susceptibility of *S. litura* to *H. indica* at different time interval (LD 50).

Time interval (h)	LD 50 (IJs/larva)	Regression equation
24	10.1	y = 2.96 ln(x) – 3.21
48	6.9	y = 2.60 ln(x) – 0.88
72	3.6	y = 2.13 ln(x) +1.80
96	1.6	y = 1.66 ln(x) +4.10

In second study, among the tested strains EPNs, *S. abbasi* (HAR-EPN-Sa-3) was found more pathogenic to *S. litura* as it brought about nearly 50 per cent mortality of the larvae at an inoculum level of 10IJs per insect larva after 24h followed by HAR-EPN-Sa-2, HAR-EPN-Sa-5 HAR-EPN-Sa-4 and HAR-EPN-Sa-1. In case of *H. indica*, strain (HAR-EPN-Hi-2) was found more pathogenic to *S. litura* as it brought about approximately 50 per cent mortality of *S. litura* at an inoculum level of 10IJs per larva.

Table 12: Compatibility of most virulent strains of EPNs (HAR-EPN-Sa-3 and HAR-EPN-Hi-2) against *S. litura*.

Treatments (IJs/petri plate)	Larval mortality at different time intervals (h)				
	24	48	72	96	Mean
T_1- Uninoculated check	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
T_2- Virulent strain of *Heterorhabditis* spp.	42.5 (40.5)	60.0 (51.1)	77.5 (62.3)	92.4 (77.3)	68.1 (57.8)
T_3- Virulent strain of *Steinernema* spp.	52.5 (46.4)	70.0 (57.1)	87.4 (71.4)	97.3 (83.2)	76.8 (64.5)
T_4- T_2+T_3	72.5 (58.6)	87.4 (71.7)	97.3 (83.2)	99.7 (87.1)	89.2 (75.2)
Mean	55.8 (37.1)	72.5 (45.7)	87.4 (54.9)	96.5 (62.6)	

Values in parenthesis are angular transformed values.
C.D. at 5% Time:- (5.7), Dosages:- (5.7), Interaction (Time v/s Dosages):- (11.3)

In this experiment, EPNs strains ((HAR-EPN-Sa-3 and HAR-EPN-Hi-2) which were more virulent against *S.litura* were used. The *S. abbasi* in combination with *H. indica* was more virulent than *S. abbasi* and *H. indica* when applied alone. Two or more EPN species often occur sympatrically, commonly infect the same host individual, and thus have the potential to compete interspecifically for a shared host resource and adversely influence each other's survival [7] [16]. The percent mortality of insect larvae increased with increase in exposure time. After 72 and 96h, both (H+S) caused 97.3 and 99.7 percent mortality. There was a mean death of 39.7 per cent and at 20IJs, 73.7 per cent mortality of *S. litura* could be recorded (Table 12). However, multiple species can coexist in an environment if they possess different foraging strategies e.g., ambushers vs. cruisers [10] exhibited different levels of host specificity, exploited different spatial niches in the soil, or occur in aggregated distributions [7] [4]. The possibility for exploitative competition between two EPNs is enhanced because there is little evidence that infective juvenile of EPN avoid hosts previously infected by another genus or species of EPN [10].

Results in present studies suggested that the inter-specific competition between steinernematid species showed that two species can co-infect a host individual, but that one EPN species ultimately prevail to reproduction [1] [7]. This idea is supported by the study of Choo [3] and Koppenhofer [9] who reported that such species specific differences in behavior and foraging niche may explain why various combinations of EPN species result in additive mortality of scarab beetle larvae suggesting weak interspecific competition in these cases. It could be concluded that *S. abbasi* when inoculated at a moderate level of 10IJs per larva could cause on an average more than 50 per cent mortality. Maximum mortality was observed at 40IJs which were significantly different from other treatments of interactions. Hussaini [6] recorded highest larval mortality of *S. litura* at an inoculum level of 100IJs of *Heterorhabditis* and 125IJs of *Steinernema spp*. In the present studies, high larval mortality of *S. litura* was observed at high inoculum level of 40IJs per larva.

Conclusion

The findings of indigenous EPN species/strains from Haryana gives some promise for laying the foundations for developing novel, non-chemical pest controls. Native isolates of biological control agents are better adapted to the local agro-climatic conditions as compared to exotic species; therefore, they are ideal for local biological insect pest management programs. This will benefit growers in areas where pesticides are not accessible and too toxic or not practical. Consumers will also benefit from foods with less pesticide residue.

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