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Research Article

In-vivo production of a Kashmir isolate of EPN, *Heterorhabditis bacteriophora* (SKUASTK-EPN-Hr 01) on test insect hosts from Srinagar (J&K)

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ABSTRACT: Under laboratory conditions, the virulence of the Kashmir isolate of EPN, *Heterorhabditis bacteriophora* (SKUASTK-EPN-Hr 01) to *Galleria mellonella*, *Corcyra cephalonica* and *Bombyx mori* showed that after 48-72 h, 100% mortality was recorded in all the test insects. Considering 48 h time and 20 IJs/larva as standard against *G. mellonella*, *C. cephalonica* and *B. mori*, the LC_{50} and LT_{50} values for the EPN were 10.17, 28.72 and 23.81 IJs/larva, and 38.64, 53.04 and 49.20 h after exposure, respectively. Again its virulence to the larvae of three different sizes of these insects, viz., small (0.12g, 0.04g, 0.17g), medium (0.18g, 0.05g, 0.59g) and large (0.22g, 0.07g, 2.58g) sized larvae showed that all the three sizes of *G. mellonella* were more susceptible than that of *B. mori* and *C. cephalonica*. Considering 20 IJs/larva as standard against small, medium and large sized larvae, the LT_{50} value for the EPN was 25.00, 26.45 and 27.21 h; 47.73, 48.92 and 53.16 h, and 46.04, 47.48 and 48.92 h for *G. mellonella*, *C. cephalonica* and *B. mori*, respectively. The production of nematode infective juveniles per larvae was directly proportional to the size and/or body weight of the insect species tested. The average production of nematode infective juveniles per gram of host body weight ranged from 1557.79 to 1933.55 x 10^3, 217.38 to 335.43 x 10^3 and 71.03 to 128.00 × 10^3 in *G. mellonella*, *C. cephalonica* and *B. mori*, respectively. The total time period between the larval mortality and the initiation of emergence, and between larval mortality and the cessation of emergence of nematode infective juveniles from the cadavers ranged from 7-10, 6-8 and 9-12 days, and 19-28, 16-24 and 23-41 days in *G. mellonella*, *C. cephalonica* and *B. mori*, respectively.

KEY WORDS: *Heterorhabditis bacteriophora, Bombyx mori, Corcyra cephalonica, Galleria mellonella*, *In-vivo* production, virulence

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INTRODUCTION

Entomo-Pathogenic Nematodes (EPNs) belonging to Steinernematidae and Heterorhabditidae have considerable potential for the biological control of a wide range of insects they contact in moist habitats, such as soil. But even very effective EPNs cannot be successfully used for the management of insect pests unless it is made readily available to the users. Recently out of 13% biopesticide sale in industrialized countries, EPNs sale was only second to *Bacillus thuringiensis* Berliner at 80% (Lisansky and Coombs, 1994). EPN products are available worldwide for control of insect pests largely due to the progress made in their mass production and now EPNs can be mass produced by *in vivo* or *in vitro* methods. Breakthroughs in production and formulation have made commercialization of EPN products possible (Georgis, 1992). Laboratory rearing of insect larvae susceptible to EPNs was found to be a simple and viable technique not only because of its easy handling procedure which could be practiced by any one, but it could easily accommodate a large number of larvae per culture (Singh, 1994). For this reason, the present study was aimed to study the virulence and laboratory culture of the Kashmir isolate of EPN, *Heterorhabditis bacteriophora* Poinar (SKUASTK-EPN-Hr 01) (Rhabditida: Heterorhabditidae) on test insect hosts.

MATERIALS AND METHODS

Three test insect species, viz., greater wax moth, *Galleria mellonella* L. (Lepidoptera: Galleridae), the rice moth, *Corcyra cephalonica* L. (Lepidoptera: Pyralidae) and the silk worm, *Bombyx mori* L. (Lepidoptera: Bombycidae) were selected as test hosts to study the virulence and laboratory production of Kashmir isolate of EPN, *H. bacteriophora* (SKUASTK-EPN-Hr 01). The *G. mellonella* and *C. cephalonica* were reared on their respective artificial diets (Singh, 1994) while the *B. mori* were reared on mulberry leaves (Zaki, 2000). Petri dish evaluation method was adopted to study the virulence of *H. bacteriophora* against the test hosts @ 0, 5, 10, 20, 30, 40

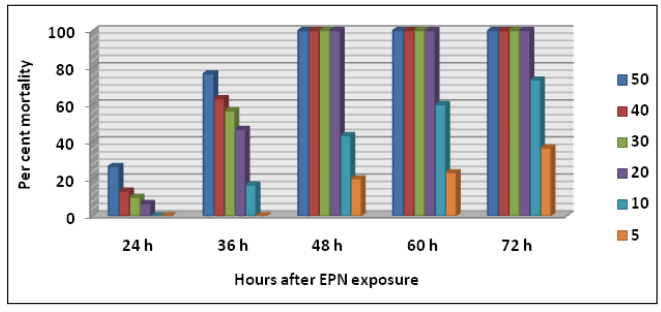
and 50 IJs/larva. The assay was carried out in 9 cm. diam. Petri dishes lined with Whatmann # 1 filter paper. There were three replications of each treatment arranged in CRD and each replicate consisted of ten insects. The Petri dishes were kept in plastic bags to conserve moisture while incubating at 24±2 °C in a BOD incubator. The observations were recorded on the host mortality up to 72 h at intervals of 12 h and then probit regression analysis was done to determine LC_{50} and LT_{50} values.

In another experiment, the infective juveniles (IJ_3) of *H. bacteriophora* (SKUASTK-EPN-Hr 01) were inoculated on three groups (large, medium, small) of these three test hosts based on their larval sizes or body weights. The average weights of large, medium and small sized larvae of *G. mellonella*, *C. cephalonica* and *B. mori* were 0.22, 0.18, and 0.12; 0.07, 0.05 and 0.04, and 2.58, 0.59 and 0.17g /larva, respectively. The EPN was diluted in an appropriate quantity of distilled water to obtain 200 IJs/ml of distilled water and 1.0 ml of the nematode suspension was evenly distributed on a Whatmann # 1 filter paper in a 9 cm diam. Petri dish. The rest of the procedure was same as given above. The infected larvae (cadavers) were washed with sterile distilled water and then placed on "White's Trap" (White, 1927) for emergence of the IJ_3. The recovery of *H. bacteriophora* from the cadavers of *G. mellonella*, *C. cephalonica* and *B. mori* was recorded separately. The initiation of emergence of IJ_3 from the cadavers was observed daily up to the cessation of emergence of infective juveniles from the cadavers.

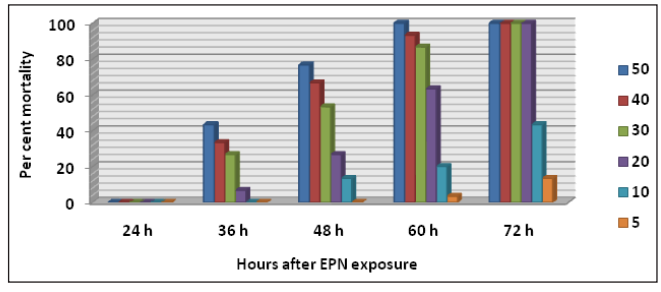
RESULTS AND DISCUSSION

The results of the virulence of the Kashmir isolate of EPN, *H. bacteriophora* (SKUASTK-EPN-Hr 01) to *G. mellonella*, *C. cephalonica* and *B. mori* showed that after 48-72 h, 100% mortality was recorded in all the test insects. Mortality increased with increase in time and nematode concentration. There was no mortality in control of the test insect species during the period of exposure in the laboratory experiment. After 48 h of exposure, there were 100.00, 26.67 and 46.67% mortality of *G. mellonella, C. cephalonica* and *B. mori* at the dosages of 20 IJs/larva, respectively (Figure 1). The data on nematode concentration factor versus mortality computed at median lethal concentration (LC_{50}) of *H. bacteriophora* against *G. mellonella, C. cephalonica* and *B. mori* were worked out to be 25.82, 52.71 and 44.12 IJs/larva at 36 h, 7.75, 15.88 and 12.35 IJs/larva at 60 h, and 6.34, 10.15 and 8.74 IJs/larva at 72 h post inoculation, respectively. The LC_{50} value for the EPN considering 48 h time as standard against *G. mellonella*, *C. cephalonica* and *B. mori* was 10.17, 28.72 and 23.81 IJs/larva, respectively (Table 1). Time assay response with the same data revealed that the median lethal time (LT_{50}) of *H. bacteriophora* against *G. mellonella, C. cephalonica* and *B. mori* were to be 28.58, 38.77 and 35.62 h at 50 IJs/larva; 31.72, 41.60 and 38.69 h at 40 IJs/larva, 32.90, 44.50 and 40.37 h at 30 IJs/larva; 54.02, 76.55 and 70.61 h at 10 IJs/larva, and 80.42, 91.71 and 96.93 h at 5 IJs/larva, respectively. Considering 20 IJs/larva as standard against *G. mellonella*, *C. cephalonica* and *B. mori*, the LT_{50} value for the EPN was 38.64, 53.04 and 49.20 h after exposure, respectively (Table 2).

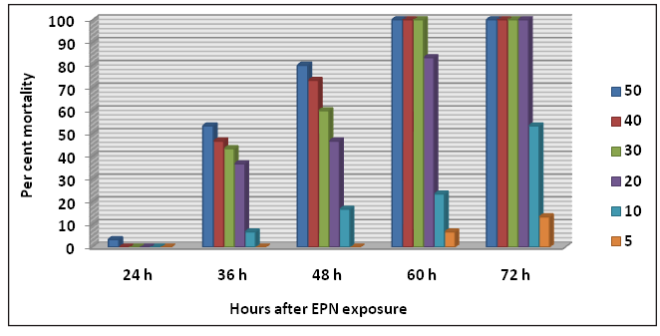
The results of virulence of the Kashmir isolate of EPN, *H. bacteriophora* to the larvae of three different sizes/weights, viz., small (0.12g, 0.04g, 0.17g), medium (0.18g, 0.05g, 0.59g) and large (0.22g, 0.07g, 2.58g) of *G. mellonella*, *C. cephalonica* and *B. mori*, respectively showed that all the three sizes/weights of *G. mellonella* were more susceptible than that of *B. mori* and *C. cephalonica*. After 48 h of exposure there was 100.00% mortality in all the three sizes/weights of *G. mellonella* while 53.33, 36.67 and 26.67%, and 73.33,



Greater wax moth, *Galleria mellonella*



Rice moth, *Corcyra cephalonica*



Silk worm, *Bombyx mori*

Fig. 1. Virulence test of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* against different insect hosts.

Table 1. **Concentration mortality response of different insect hosts to Kashmir isolate of EPN, *Heterorhabditis bacteriophora* in Srinagar**

Insect host	Parameter (IJs/larva)	Post treatment (Hours after exposure)				
		24	36	48	60	72
Galleria mellonella	LC_{50}	92.41	25.82	10.17	7.75	6.34
	SD	2.994	8.786	10.815	9.683	7.791
Corcyra cephalonica	LC_{50}	-	52.71	28.72	15.88	10.15
	SD	-	5.576	9.217	12.176	11.461
Bombyx mori	LC_{50}	-	44.12	23.81	12.35	8.74
	SD	-	6.683	9.558	12.769	11.003

Total number of insects = 30; Dose = 20 IJs/larva; SD= Standard deviation, LC_{50}= Median lethal concentration (IJs/larva) to kill 50% test insects

Table 2. **Time mortality response of different insect hosts to Kashmir isolate of EPN, *Heterorhabditis bacteriophora* in Srinagar**

Insect host	Parameter (HAE)	Post treatment (IJs/larva)					
		5	10	20	30	40	50
Galleria mellonella	LT_{50}	80.42	54.02	38.64	32.90	31.72	28.58
	SD	4.764	9.071	12.774	12.020	11.436	9.549
Corcyra cephalonica	LT_{50}	91.71	76.55	53.04	44.50	41.60	38.77
	SD	1.732	5.366	12.577	12.409	12.601	12.794
Bombyx mori	LT_{50}	96.93	70.61	49.20	40.37	38.69	35.62
	SD	1.788	6.204	11.853	12.617	12.617	12.173

Total number of insects = 30; Dose = 20 IJs/larva; SD = Standard deviation, LT_{50}= Median lethal time (Hours after exposure) to kill 50% test insects, HAE= Hours after exposure

56.67 and 36.67% mortality of small, medium and large sized/weighted larvae of *C. cephalonica* and *B. mori* at the dosages of 20 IJs/larva, respectively. Considering 20 IJs/larva as standard against small, medium and large sized/weighted larvae of *G. mellonella*, *C. cephalonica* and *B. mori*, the LT_{50} value for the EPN was 25.00, 26.45 and 27.21 h, 47.73, 48.92 and 53.16 h and 46.04, 47.48 and 48.92 h, respectively (Table 3). *G. mellonella* was found most preferred host for the EPN as it was killed earlier than *B. mori* and *C. cephalonica*. The size of peritrophic membrane in the three test insect larvae appears to be a factor to differences in EPN parasitism. Similar results were reported by Forschler and Gardner (1991) for nematode entry in case of *G. mellonella*. Differences in infectivity among *G. mellonella*, *C. cephalonica* and *B. mori* by *H. bacteriophora* may also result from their volatile cues in nature and protein-lipid ratios in the test insect hosts. Such a relative susceptibility of insect species to the EPNs has also been reported earlier by Kim *et al*. (1995), Zaki *et al*. (2000) and Subramanian (2003).

The EPN, *H. bacteriophora* was successfully cultured on all the three sizes of all the three insect species. The results on the yield of *H. bacteriophora* from the three test insect species revealed that the production of IJ_3 per larvae was directly proportional to the size and/or body weight of the insect species tested. The average production ranged from 145.43 to 170.85 x 10^3, 235.50 to 240.47 x 10^3 and 289.37 to 334.10 x 10^3 IJs; 6.34 to 6.59 x 10^3, 10.60 to 11.08 x 10^3 and 18.98 to 19.30 x 10^3 IJs, and 11.29 to 15.74 x 10^3, 37.97 to 43.76 x 10^3 and 101.92 to 120.24 x 10^3 IJs in small, medium and large sized larvae of *G. mellonella*, *C. cephalonica* and *B. mori* with mean larval body weights of 0.10, 0.14 and 0.17; 0.03, 0.04 and 0.06, and 0.10, 0.40 and 1.56 g, respectively. Overall the average production of IJ_3 per gram of host body weight ranged from 1557.79 to 1933.55 x 10^3, 217.38 to 335.43 x 10^3 and 71.03 to 128.00 x 10^3 in *G. mellonella*, *C. cephalonica* and *B. mori*, respectively. While computing the pooled mean production of IJ_3, a yield of 1744.17 x 10^3, 274.31 x 10^3 and 100.67 x 10^3 IJs per gram of host larval body weight in *G. mellonella*, *C. cephalonica* and *B. mori* was recorded, respectively (Table 4) indicating, thereby, that among the three insects, *G. mellonella* yielded the highest IJ_3 per gram of host larval body weight followed by *C. cephalonica* and lowest in *B. mori*. The number of EPNs emerging from the cadavers of insect hosts appears to be dependent on the size and/or body weight of the test hosts. In the large sized larvae the total quantity of *nutrient soup* available for the nematodes was relatively more, which could support maximum production of EPNs and consequently there was no competition for food and space and as a result of this, a large number of nematodes were produced. The comparative lesser production of EPNs in small sized larvae

Table 3. **Time mortality response of size of different insect host larvae to Kashmir isolate of EPN, *Heterorhabditis bacteriophora* in Srinagar**

Size of insect larvae	Post treatment					
	Galleria mellonella		*Corcyra cephalonica*		*Bombyx mori*	
	LT_{50} (HAE)	SD	LT_{50} (HAE)	SD	LT_{50} (HAE)	SD
Small	25.00	11.547	47.73	15.011	46.04	15.534
Medium	26.45	15.011	48.92	15.176	47.48	15.044
Large	27.21	16.165	53.16	15.534	48.92	15.176

Total number of insects = 30; Dose = 20 IJs/larva; SD = Standard deviation, LT_{50} = Median lethal time (Hours after exposure) to kill 50% test insects, HAE= Hours after exposure

Table 4. **Effect of size/weight of different insect host larvae on the laboratory production of Kashmir isolate of EPN, *Heterorhabditis bacteriophora***

Insect species	Size of insect larvae	Avg. weight of larva (g)		No. of IJs produced/larva (x 10^3)			No. of IJs produced/g body weight larva (x 10^3)		1st emergence period (DAM)	Peak emergence (DAM)	Cessation period (DAM)
		Live	Dead	Min.	Max.	Avg.	Avg.	Mean			
Galleria mellonella	Small	0.12	0.10	145.43 (5.16)	170.85 (5.23)	158.74 (5.20)^{c}	1557.79 (6.19)^{c}	1744.17 (6.24)	7-9	12-15	19-24
	Medium	0.18	0.14	235.50 (5.37)	240.97 (5.38)	238.03 (5.38)^{b}	1741.18 (6.24)^{b}		8-9	14-17	22-25
	Large	0.22	0.17	289.37 (5.46)	334.10 (5.52)	326.16 (5.51)^{a}	1933.55 (6.29)^{a}		9-10	15-19	25-28
Corcyra cephalonica	Small	0.04	0.03	6.34 (3.80)	6.59 (3.82)	6.47 (3.81)^{i}	217.38 (5.34)^{f}	274.31 (5.43)	6-7	10-12	16-19
	Medium	0.05	0.04	10.60 (4.02)	11.08 (4.04)	10.81 (4.03)^{h}	270.13 (5.43)^{e}		6-8	12-16	18-22
	Large	0.07	0.06	18.98 (4.28)	19.30 (4.28)	19.12 (4.28)^{f}	335.43 (5.52)^{d}		7-8	13-16	19-24
Bombyx mori	Small	0.17	0.10	11.29 (4.05)	15.74 (4.19)	13.19 (4.12)^{g}	128.00 (5.11)^{g}	100.67 (4.99)	9-10	12-14	23-27
	Medium	0.59	0.40	37.97 (4.58)	43.76 (4.64)	40.25 (4.60)^{e}	102.97 (5.01)^{h}		10-11	14-17	30-33
	Large	2.58	1.56	101.92 (5.01)	120.24 (5.08)	110.53 (5.04)^{d}	71.03 (4.85)^{i}		11-12	16-20	38-41
C.D. (P=0.05)		**-**	**-**	**(0.07)**	**(0.05)**	**(0.06)**	**(0.03)**	**-**	**-**	**-**	**-**

Each figure is mean of three replicates containing 10 insect larvae each; Figures in parentheses are log transformed values; Figures in columns followed by common letter(s) do not differ significantly from one another at 5% level of significance; DAM: Days after mortality

may probably be due to not only of less food availability but also of completion of lesser number of generations by the time of food is exhausted. Our results are in consistent with the findings of Sivakumar *et al*. (1988) who have obtained 17108 ± 856 IJs of *H*. *bacteriophora* from a larva of *C*. *cephalonica*. Ali *et al*. (2005) have reported a recovery of 0.5-2 x 10^5 IJs/larva from *C*. *cephalonica* at Kanpur. Zaki *et al*. (2000) reported that nematode emergence of exotic strain of *H*. *bacteriophora* from the cadavers of *B*. *mori* with an average of 2,750 IJs/larva in Kashmir.

The IJ_3's emergence started at 7-9, 8-9 and 9-10; 6-7, 6-8 and 7-8, and 9-10, 10-11 and 11-12 days after mortality (DAM) and the total progeny emergence period continued from 19-24, 22-25 and 25-28; 16-19, 18-22 and 19-24, and 23-27, 30-33 and 38-41 DAM in case of small, medium and large sized larvae of *G*. *mellonella*, *C*. *cephalonica* and *B*. *mori* larvae, respectively. The mean duration of nematode emergence from cadavers of three insect hosts differed significantly among one another. The duration of nematode emergence was shorter in small sized cadavers of *G*.

mellonella (12-15 days), *C. cephalonica* (10-12 days) and *B. mori* (14-17 days) as compared to that of large sized cadavers (15-19, 13-16 and 16-20 days, respectively) (Table 4). The reason may be that in small sized larvae of *G. mellonella*, *C. cephalonica* and *B. mori*, the food for nematode's growth and reproduction exhausted very fast and nematodes were forced to exit from the cadavers earlier. Contrarily to it, in large sized larvae of *G. mellonella*, *C. cephalonica* and *B. mori*, the food for nematode's growth and reproduction did not exhaust very fast and nematodes were not forced to exit from the cadavers earlier. Our results are in agreement with that of Flanders *et al*. (1996) and Karunakar *et al*. (2000) who have reported a delay in nematode emergence from the larger larvae and that the number of IJ_3 were also more in larger larvae than smaller larvae. Zaki *et al*. (2000) reported that the duration of nematode emergence of *H. bacteriophora* up to 20 days after emergence from the cadavers of *B. mori*. The results indicated that all the three insect hosts offer good potential for laboratory production of Kashmir isolate of EPN, *H. bacteriophora*. For the purpose of better production, it is ideal to inoculate large sized larvae of *G. mellonella*, *C. cephalonica* or *B. mori*. Moreover, amongst the three insect hosts tested, *G. mellonella* was found most suitable for laboratory production of EPNs and it can be obtained directly from the rearing room at low cost.

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