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Research Article

Virulence and recovery of a cold tolerant Kashmir isolate of EPN, *Heterorhabditis bacteriophora* (SKUASTK-EPN-Hr 01) with respect to white grub, *Heteronychus* sp. in Srinagar (J and K)

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ABSTRACT: The virulence of a cold tolerant indigenous Kashmir isolate of *Heterorhabditis bacteriophora* (SKUASTK-EPN-Hr 01) tested against 1st, 2nd and 3rd instars of white grub, *Heteronychus* sp. @ 0, 100, 150, 200, 250, 500, 750 and 1000 IJs/grub using Petri dish bioassay method, revealed large differences in the virulence of *H. bacteriophora* towards the different stages of *Heteronychus* sp. However, there was a positive correlation between nematode concentration and the insect mortality. The 3rd instar grubs succumbed to nematode infection later (LT_{50} = 6.47 days at 250 IJs/grub) than the grubs of 2nd (LT_{50} = 3.23 days at 250 IJs/grub) and 1st instars (LT_{50} = 2.44 days at 250 IJs/grub). When grubs were in 1st instar, relatively shorter exposure periods and low nematode concentrations (LC_{50} = 126.72 IJs/grub at 5 DAE) were needed for achieving lethal nematode infections as compared to 2nd (LC_{50} = 176.02 IJs/grub at 5 DAE) and 3rd (LC_{50} = 456.35 IJs/grub at 5 DAE) instar grubs where longer exposure periods and high nematode concentrations were required. The recovery of nematode infective juveniles per grub ranged from 47.47-51.32 x 10^3, 89.19-92.85 x 10^3 and 260.36-263.95 x 10^3 IJs in 1st, 2nd and 3rd larval instars of *Heteronychus* sp., respectively. The total time period between the grub mortality and the initiation of emergence and between grub mortality and the cessation of emergence of nematode infective juveniles from the cadavers ranged from 6-8, 14-16 and 22-24 days, and 21-26, 31-36 and 42-45 days in case of 1st, 2nd and 3rd larval instars of white grub, *Heteronychus* sp., respectively.

KEY WORDS: Concentration, mortality response, nematode recovery, *Heterorhabditis bacteriophora, Heteronychus* sp.

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INTRODUCTION

India takes pride in being one of the world's leading producers of horticultural and agricultural commodities. The protection of agricultural crops from various kinds of pests is an important aspect of crop production. Biological control is regarded as more beneficial than pesticide-based control due to the ecological advantages such as safety to beneficial organisms, wide host range, etc. (Divya and Sankar, 2009). Biopesticides are advantageous due to their target specificity, eco-safety, no development of resistance, reduced number of applications, yield and quality improvement, higher acceptability, etc. The annual growth in pesticides use is 1-2% and that of biopesticides is 10-25% (Ahmad and Leather, 1994). Entomopathogenic Nematodes (EPNs) are one of the viable biopesticides for insect pest control available today. The native EPN strains are adapted to local conditions and are considered to be the ideal candidates for regional biological control programme (Zaki *et al*., 2000). In J and K state, there has been a strong concern over the bio safety of environment by discouraging the use of chemical pesticides in crop protection and the present study was aimed to use the Kashmir isolate of EPN, *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) against white grub, *Heteronychus* sp. (Coleoptera: Scarabaeidae: Dynastinae management) in order to minimize the use of pesticides in the crop protection technology.

MATERIALS AND METHODS

The culture of the local entomopathogenic nematode isolate, *Heterorhabditis bacteriophora* (SKUASTK-EPN-Hr 01) was maintained in the Biological Control Laboratory, Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar following the standard procedures and protocols. The white grub, *Heteronychus* sp. grubs were collected from the turf grass of Royal Spring Golf Course, Srinagar by digging with a spade/khurpi and hand shifting the soil for grub culture and was maintained in the laboratory. The 1st, 2nd and 3rd instar

grubs were placed instar wise in plastic containers along with some soil and roots. In the laboratory, only uninjured grubs were maintained in earthen pots planted with lawn grass, *Cynodon dactylon* Linnaeus (Poales: Poaceae) and caged with aluminium mesh to check the exit of grubs from the pots. The 1st, 2nd and 3rd stage grubs were selected for the study and Petri dish evaluation method was adopted to study the virulence of entomopathogenic nematode, *H. bacteriophora* against 1st, 2nd and 3rd instars of white grub, *Heteronychus* sp. @ 0, 100, 150, 200, 250, 500, 750 and 1000 IJs/grub. 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 daily on the host mortality up to 7-10 days and then probit regression analysis was done to determine LC_{50} and LT_{50} values. The recovery of *H. bacteriophora* from the cadavers of 1st, 2nd and 3rd instars of *Heteronychus* sp. was studied. The initiation of emergence of infective juveniles from the cadavers was observed daily up to the cessation of emergence of infective juveniles from the cadavers.

RESULTS AND DISCUSSION

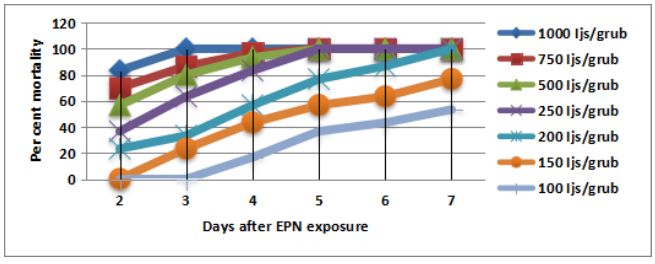
The sluggishness of *Heteronychus* sp. grubs upon exposure to the infective juveniles of Kashmir isolate of EPN, *H. bacteriophora* was observed in Petri dishes and it was found that the grubs had chewed most part of the water-treated (control) as well as the nematode-treated Whatmann filter papers. So, it was presumed that the nematode infective juveniles were also ingested by the grubs. The sluggishness of *Heteronychus* sp. grubs observed in Petri dishes might have facilitated the host attachment of *H. bacteriophora* infective juveniles and subsequent infection. The nematode infective juveniles were observed over the entire surface of *Heteronychus* sp. grubs suggesting that the nematode had no apparent affinity for any area or region of the body of grubs. The nematode infective juveniles might have used any route (oral, anal, spiracles, cuticle) of entry for infecting *Heteronychus* sp. grubs. Our results are in agreement with that of Forschler and Gardner (1991) who eliminated the role of non attraction of *H. heliothidis* Khan, Brooks and Hirschmann (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) to the grubs of *Phyllophaga hirtula* (Coleoptera: Scarabaeidae) and has demonstrated no preference by the nematodes for any specific body region of the grubs.

There was no mortality of 1st, 2nd and 3rd instars of white grub in control during the period of the laboratory experiment. All the three grub stages of *Heteronychus* sp. were found susceptible to the Kashmir isolate of EPN, *H. bacteriophora* infection following the exposure of grubs to nematode infective juveniles on filter paper (Plate 1). An indirect relationship was recorded between larval instars and larval susceptibility to *H. bacteriophora*. However, there was a positive correlation between nematode concentration and the insect mortality (Fig. 1). Experiment on nematode concentration factor versus mortality of *Heteronychus* sp. grubs computed at the median lethal concentration (LC_{50}) of the EPN, *H. bacteriophora* against 1st 2nd and 3rd instar grubs of *Heteronychus* sp. were worked out to be 440.11, 780.55 IJs/grub at 2 days, 247.44, 409.68 IJs/grub at 3 days, 170.08, 225.94 and 1033.59 IJs/grub at 4 days, 126.72, 176.02 and 456.35 IJs/grub at 5 days, 115.32, 134.54 and 308.23 IJs/grub at 6 days and 94.87, 106.38 and 231.93 IJs/grub at 7 days post inoculation (Table 1). In general, the LC_{50} values ranged from 170.08 to 94.87, 225.94 to 106.38 and 1033.59 to 231.93 IJs/grub at 4-7 days for 1st, 2nd and 3rd instar grubs of *Heteronychus* sp. using Petri dish bioassay method, respectively. The LC_{50} value for the EPN considering 4 days time as standard against 1st 2nd and 3rd instar grubs of *Heteronychus* sp. was 170.08, 225.94 and 1033.59 IJs/grub, respectively (Table 1).

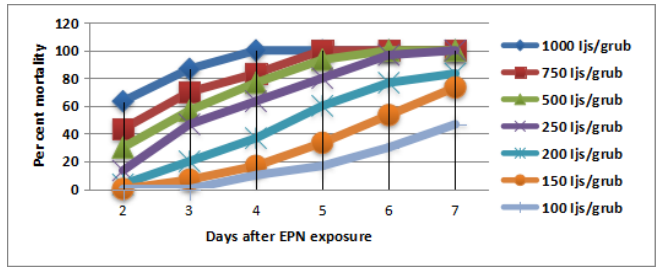
Time assay response with the same data revealed that the median lethal time (LT_{50}) of the EPN, *H. bacteriophora* against 1st, 2nd and 3rd larval instars of white grub, *Heteronychus* sp. were to be 1.48, 1.81 and 4.09 days at 1000 IJs/grub, 1.62, 2.27 and 4.33 days at 750 IJs/grub, 1.93, 2.67 and 4.92 days at 500 IJs/grub, 2.44, 3.23 and 6.47 days at 250 IJs/grub, 3.35, 4.48 and 7.79 days at 200 IJs/grub, 4.71, 5.64 and 9.57 days at 150 IJs/grub and 6.35, 7.25 and 10.59 days at 100 IJs/grub, respectively (Table 2). In general, the LT_{50} values ranged from 6.35 to 1.48 days, 7.25 to 1.81 days and 10.59 to 4.09 days at 100-1000 IJs/grub of 1st, 2nd and 3rd instar grubs of *Heteronychus* sp. using Petri dish bioassay method, respectively. Considering 250 IJs/grub as standard against 1st, 2nd and 3rd larval instars of white grub, *Heteronychus* sp. the LT_{50} value for the EPN was 2.44, 3.23 and 6.47 days after exposure (Table 2). The results strongly suggested that *Heteronychus* sp. is susceptible to the Kashmir isolate of EPN, *H. bacteriophora* during the grub stage. The variability in nematode virulence against different stages that we experienced might have been due to differences in mechanisms by which different white grub instars were able to resist the nematode attack. Several studies on the relative virulence of EPNs to *Holotrichia serrata* (Fabricius) (Coleoptera: Scarabaeidae) (Prabhuraj *et al*., 2003) and *Phyllophaga georgiana* Horn (Coleoptera: Scarabaeidae) (Koppenhofer *et al*., 2008) have reported similar conclusions. Our results together with the above studies provide further support to the hypothesis that the Kashmir isolate of EPN, *H. bacteriophora* has the ability not only to overcome the host

defenses but are also well adapted to parasitize scarabaeid larvae, which are otherwise very hardy to chemical treatments and other pathogens (Mohiuddin *et al*., 2006). Previously, some exotic isolates of *H. indica* Poinar, Karunakar and David (Rhabditida: Heterorhabditidae) from NBAIR, Bangalore had been evaluated against *Heteronychus* sp. in Kashmir but with no success (Wani, 2010). However, the findings also stress the need for application of entomopathogenic nematodes in the field when the grubs are in the early instars to achieve the good results.

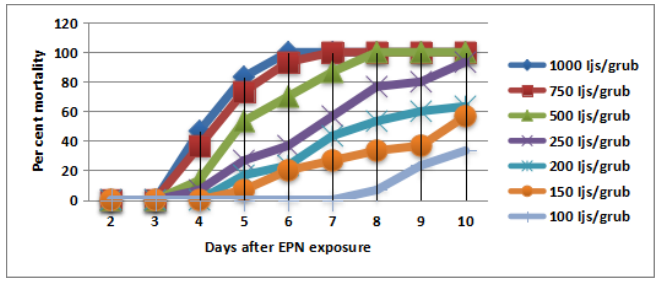
The nematode, *H. bacteriophora* was successfully cultured on the white grub, *Heteronychus* sp. (Plate 2). However, there were significant differences on the number of nematode infective juveniles produced among the three larval instars. The results on the yield of *H. bacteriophora* from the three test larval instars revealed that the recovery of nematode infective juveniles per grub was directly proportional to the size and/or body weight of the larval instars tested. Overall the average recovery of nematode infective juveniles per grub was found to be 49.98 x 10^3, 91.95 x 10^3 and 262.56 x 10^3 and that from per gram of host body weight was 357.80, 303.13 and 271.61 x 10^3 in 1st, 2nd and 3rd larval instars of white grub, *Heteronychus* sp., respectively (Table 3). The total time period between the larval mortality and the initiation of emergence of the nematode infective juveniles from the cadavers ranged from 6-8, 14-16 and 22-24 days in case of 1st, 2nd and 3rd larval instars of white grub, *Heteronychus* sp., respectively (Table 3). The peak period of emergence of nematode infective juveniles from the cadavers of 1st, 2nd and 3rd larval instars of white grub, *Heteronychus* sp. ranged from 5-8, 6-9, 6-12 days after mortality, respectively (Table 3). The results suggested that *Heteronychus* sp. can also be considered for the production of *H. bacteriophora* in the laboratory as the former is found in abundance in the golf courses and fields of Kashmir valley. The present studies confirm the observations of Jat and Choudhary (2006) who had harvested 16,800 IJs/grub of 1st instar of *H. consanguinea* Blanchard (Coleoptera: Scarabaeidae) and Karunakar *et al*. (2000a) who have reported 24,128 IJs/grub of *S. glaseri* Steiner (Rhabditida: Steinernematidae) and 71,923 IJs/grub of *H. indica* in the grub *H. serrata*. The duration of nematode emergence recorded, was 21-26, 31-36 and 42-45 days after mortality in case of 1st, 2nd and 3rd larval instars of white grub, *Heteronychus* sp., respectively. The early duration of nematode emergence from the larval body of 1st instar might be due to food exhaustion for nematode's growth and reproduction owing to its smaller size and hence were forced to exit from the cadavers early. Contrary to it, 2nd and 3rd instar grubs, because of their bigger size offered more food for sustained multiplication of nematodes. Our results are in agreement with that of Flanders *et al*. (1996) and Karunakar *et al*. (2000b) who have reported a delay in nematode emergence from the larger cadavers and that the number of infective juveniles were also more in larger cadavers in comparison to that of smaller cadavers.



1st instar grubs



2nd instar grubs



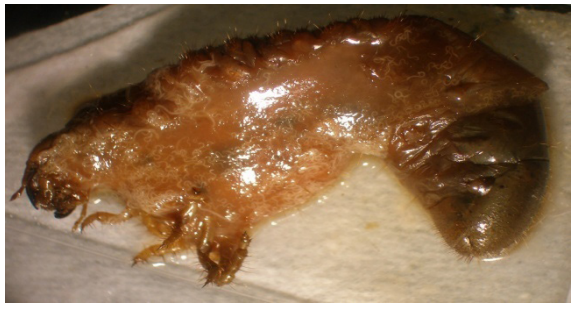
3rd instar grubs

Fig. 1. Laboratory evaluation of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* against different instar grubs of *Heteronychus* sp. using Petri dish bioassay method.

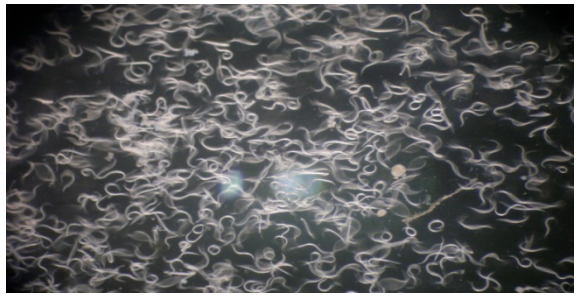
3rd instar healthy white grub

3rd instar EPN infected white grubs (Cadavers)

Plate 1. Virulence of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* on white grub, *Heteronychus* sp. in Kashmir



Adults and infective juveniles of *Heterorhabditis bacteriophora* feeding in 3rd instar white grub



Infective juveniles of *Heterorhabditis bacteriophora* in SDW

Plate 2. Recovery of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* from white grub, *Heteronychus* sp. in Srinagar, Kashmir

Table 1. Concentration mortality response of different larval instars of white grub, *Heteronychus* sp. to Kashmir isolate of EPN, *Heterorhabditis bacteriophora* using Petri dish bioassay method

Grub stage	Parameter (IJs/grub)	Post treatment (Days after exposure)								
		2	3	4	5	6	7	8	9	10
1st	LC_{50}	440.11	247.44	170.08	126.72	115.32	94.87	-	-	-
	SD	9.897	11.103	9.539	7.764	6.827	5.507	-	-	-
2nd	LC_{50}	780.55	409.68	225.94	176.02	134.54	106.38	-	-	-
	SD	7.367	9.877	10.390	10.045	8.395	6.027	-	-	-
3rd	LC_{50}	-	-	1033.59	456.35	308.23	231.93	184.87	164.25	135.93
	SD	-	-	5.769	9.906	11.657	11.557	11.171	9.623	8.079

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

Table 2. Time mortality response of different larval instars of white grub, *Heteronychus* sp. to Kashmir isolate of EPN, *Heterorhabditis bacteriophora* using Petri dish bioassay method

Grub stage	Parameter	Post treatment (IJs/grub)						
		100	150	200	250	500	750	1000
1st	LT_{50} (DAE)	6.35	4.71	3.35	2.44	1.93	1.62	1.48
	SD (Hours)	6.833	8.447	9.108	7.782	5.205	3.614	2.041
2nd	LT_{50} (DAE)	7.25	5.64	4.48	3.23	2.67	2.27	1.81
	SD (Hours)	5.492	8.846	9.591	9.899	8.424	6.853	4.460
3rd	LT_{50} (DAE)	10.59	9.57	7.79	6.47	4.92	4.33	4.09
	SD (Hours)	4.111	5.736	7.244	9.511	9.727	7.174	6.027

Total number of insects = 30; DAE = Days after exposure; SD = Standard deviation
LT_{50} = Median lethal time (Days after exposure) to kill 50% test insects

Table 3. Recovery of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* from white grub, *Heteronychus* sp. in Srinagar

Stage of insect grub	Avg. weight of dead grub (g)	No. of IJs produced/grub (x 10^3)			No. of IJs produced/g body weight grub (x 10^3)	1st emergence (DAM)	Peak emergence (DAM)	Cessation period (DAM)
		Min	Max	Avg				
1st	0.14	47.47 (4.68)	51.32 (4.71)	49.98 (4.70)[c]	357.80 (5.55)[a]	6-8	11-16	21-26
2nd	0.30	89.19 (4.95)	92.85 (4.97)	91.95 (4.96)[b]	303.13 (5.48)[b]	14-16	20-25	31-36
3rd	0.97	260.36 (5.41)	263.95 (5.42)	262.56 (5.42)[a]	271.61 (5.43)[c]	22-24	28-36	42-45
C.D. (P=0.05)		**3.98 (0.02)**	**4.86 (0.02)**	**5.39 (0.02)**	**2.12 (0.02)**	-	-	

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

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