

Journal of Biological Control, 35(1): 6-11, 2021, DOI: 10.18311/jbc/2021/23101

Research Article

Bioefficacy and mass culturing of *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae) on cabbage butterfly, *Pieris brassicae* (Lepidoptera: Pieridae) in temperate region of Kashmir, India

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ABSTRACT: A laboratory investigation was carried out to assess the bioefficacy and mass culturing of Kashmir isolate of entomopathogenic nematode (EPN), *Heterorhabditis bacteriophora* on cabbage butterfly, *Pieris brassicae* in Srinagar. The pathogenicity of *H. bacteriophora* against 3rd, 4th and 5th larval instars of *P. brassicae* showed cent per cent mortality of the pest in all the three larval instars at the dosages of 20 IJs/larva and above. The LC_{50} values of *H. bacteriophora* against 3rd, 4th and 5th larval instars of *P. brassicae* ranged from 46.61 to 9.32, 76.33 to 9.82 and 91.36 to 10.08 IJs/larva at 24 to 96 h and that the LT_{50} values from 65.15 to 23.12, 84.54 to 29.20 and 105.36 to 33.52 h at 10 to 60 IJs/larva, respectively. The average production of nematode infective juveniles per larvae was found to be 7.02 x 10^3, 25.74 x 10^3 and 58.45 x 10^3 and that from per gram of host body weight was 91.49 x 10^3, 151.30 x 10^3 and 233.78 x 10^3 in 3rd, 4th and 5th larval instars of *P. brassicae*, respectively. The total time period between the larval mortality and the initiation of emergence of the infective juveniles from the cadavers ranged from 7-8, 9-10 and 10-11 days and the peak period of emergence of infective juveniles ranged from 10-13, 14-19, 16-21 days after mortality from the cadavers of 3rd, 4th and 5th larval instars of *P. brassicae*, respectively. The total duration of nematode emergence recorded during the period between larval mortality and cessation of emergence of infective juveniles from the cadavers ranged 17-19, 23-25 and 26-29 days after mortality in case of 3rd, 4th and 5th larval instars of *P. brassicae*, respectively.

KEY WORDS: Bioefficacy, entomopathogenic nematode, *Heterorhabditis bacteriophora, Pieris brassicae*

(Article chronicle: Received: 06-01-2020; Revised: 14-09-2020; Accepted: 05-11-2020)

INTRODUCTION

Pest management in agriculture is a challenging task in the context of increasing agricultural productivity without disturbing the ecological balance and environment. Biological control is regarded as more beneficial than pesticide-based control due to the ecological advantages such as safety to environment, food chain, etc. (Divya and Sankar, 2009). The annual growth in pesticides use is 1-2% and that of biopesticides is 10-25% (Ahmad and Leather, 1994). The entomopathogenic nematodes in the families of Steinernematidae and Heterorhabditidae are potential biocontrol agents due to their symbiotic association with bacteria *Xenorhabdus* sp. and *Photorhabdus* sp., respectively (Woodring and Kaya, 1988; Gaugler and Kaya, 1990). The cabbage butterfly, *Pieris brassicae* is found throughout Kashmir and is a perennial problem on vegetables especially cabbage, cauliflower and knol kohl. The entomopathogenic nematode, *Heterorhabditis bacteriophora* can be used effectively to manage *P. brassicae* infesting vegetables. In Jammu and Kashmir State, there has been a strong concern over the bio safety of environment and discourages the use of chemical pesticides in crop protection. Hence, the present study was aimed to evaluate the native isolates of entomopathogenic nematode, *H. bacteriophora* against cabbage butterfly for its management.

MATERIALS AND METHODS

The larvae of cabbage butterfly, *Pieris brassicae* were collected from the field and brought to laboratory for rearing. The collected larvae were placed in the glass jars (23 cm x 10 cm) under controlled atmospheric conditions at Biological Control Laboratory of the Division of Entomology, Shalimar Campus, Srinagar. The larvae were reared on fresh, unsprayed leaves of cabbage (Golden Acre)/cauliflower (Snow ball-16) collected from the field. The feed was changed after every 24 h and the jars were simultaneously cleaned thoroughly to prevent contamination. The 3rd, 4th and 5th larval instars of the *P. brassicae* were selected for the study.

The entomopahtogenic nematode isolates were collected from Srinagar in Kashmir and evaluated against cabbage butterfly, *P. brassicae*. The isolate was multiplied in the laboratory and sent to ICAR-NBAIR for identification. The entomopathogenic nematode was idenitfied as *Heterorhabditis bacteriophora*. This *H. bacteriophora* isolate was evaluated against *P. brassicae* larvae.

Petri dish evaluation method was adopted to study the pathogenicity of entomopathogenic nematode, *H. bacteriophora* against 3rd, 4th and 5th larval instars of cabbage butterfly, *P. brassicae*. For that 1 ml of sterile distilled water containing 0, 10, 20, 30, 40, 50 and 60 IJs/larva was evenly distributed on a 9 cm diam. Whatmann # 1 filter paper in a lid of a 10 cm Petri dish. The larvae were then released on to this 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 22 ± 2°C in a BOD incubator. The observations were recorded daily on the host mortality up to 4 days and then probit regression analysis was done to determine LC_{50} and LT_{50} values. The laboratory production of entomopathogenic nematode, *H. bacteriophora* from the cadavers of 3rd, 4th and 5th larval instars of cabbage butterfly, *P. brassicae* 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 cabbage butterfly, *Pieris brassicae* was found highly susceptible to Entomopathogenic Nematode (EPN), *Heterorhabditis bacteriophora* (Plate 1). All the treatments showed a positive correlation between nematode inoculum dose and the insect mortality. There was no mortality of 3rd, 4th and 5th larval instars of *P. brassicae* in control during the period of laboratory experiment. After 24 h of exposure, there was 63.33, 50.00 and 43.33%; 30.00, 26.67 and 13.33% and 16.67, 13.33 and 6.67% mortality of 3rd, 4th and 5th larval instars of *P. brassicae* at the dosages of 60, 50 and 40 IJs/larva, respectively. After 48 h of exposure there was 100.00, 73.33 and 63.33% mortality of 3rd, 4th and 5th larval instars in a dose of 40 IJs/larva or above, respectively. Similarly, after 72 and 96 h of exposure there was 100.00% mortality in the dosages of 20, 30 and 30 IJs/larva or above while 53.33 and 73.33%; 40.00 and 56.67%, and 36.67 and 46.67% mortality of 3rd, 4th and 5th larval instars of *P. brassicae* at 10 IJs/larva, respectively (Table 1).

Experiment on nematode concentration factor versus mortality of *P. brassicae* larvae computed at median lethal concentration (LC_{50}) of EPN, *H. bacteriophora* against 3rd, 4th and 5th larval instars of *P. brassicae* was worked out to be 46.61, 76.33 and 91.36 IJs/larva at 24 h, 12.53, 18.92 and 29.02 IJs/larva at 48 h, 9.91, 11.05 and 11.73 IJs/larva at 72 h and 9.32, 9.82 and 10.08 IJs/larva at 96 h post inoculation, respectively (Table 2). The LC_{50} value for the EPN considering 48 h time as standard against 3rd larval instars of *P. brassicae* was 12.53, 18.92 and 29.02 IJs/larva.

Time assay response with the same data revealed that the median lethal time (LT_{50}) of *H. bacteriophora* against 3rd, 4th and 5th larval instars of *P. brassicae* was observed to be 23.12, 29.20 and 33.52 h at 60 IJs/larva, 24.39, 36.42 and 40.46 h at 40 IJs/larva, 34.48, 47.61and 50.24 h at 20 IJs/larva and 65.15, 84.54 and 105.36 h at 10 IJs/larva, respectively (Table 3). Considering 20 IJs/larva as standard, the LT_{50} value for the EPN against 3rd, 4th and 5th larval instars of *P. brassicae* was 34.48, 47.61and 50.24 h after exposure, respectively.

From the results it is clear that all the larval instars of *P. brassicae* tested were susceptible to the EPN, *H. bacteriophora*. With age, the larvae needed longer exposure periods to attain cent per cent mortality and the post inoculation longevity of the larvae also increased. This difference in susceptibility of *P. brassicae* larval stages to *H. bacteriophora* might be due to the differences in larval size, larval weight, larval age, physiological factor or other intrinsic factors. The variability in nematode virulence that we experienced may also have been due to a combination of minor differences in nematode storage duration, insect quality and bioassay arenas. Larval mortality may also be presumed to be related to the number of viable nematodes ingested by the insect during feeding, or infection could take place by invasion of the nematodes through the natural openings/cuticle of the insect with an undetermined minimum number required for mortality to occur. Kaya (1985) attributed the differential susceptibility of lepidopterous insect's life stages to the differences in number of portals of entry available for invading nematodes. Our data support the findings of Khan *et al*. (2007) who reported that at 25 IJs/larva, the initial and cent per cent mortality of *P. brassicae* by *Steinernema masoodi* EPN-1 was obtained at 60 and 156 h after inoculation, respectively.

The nematode, *H. bacteriophora* was successfully cultured on 3rd, 4th and 5th larval instars of *P. brassicae*. However, there were significant differences on the number of nematode infective juveniles produced among the three larval instars tested. The production of entomopathogenic nematode infective juveniles per larva ranged from 6.62 to 7.19 x 10^3, 24.43 to 26.16 x 10^3 and 57.71 to 59.42 x 10^3 in 3rd, 4th and 5th larval instars of *P. brassicae* with mean body

weight of 0.08, 0.17 and 0.25 g/larva, respectively. Overall the average production of entomopathogenic nematode infective juveniles per larva was found to be 7.02 x 10^3, 25.74 x 10^3 and 58.45 x 10^3 and that from per gram of host body weight was 91.49, 151.30 and 233.78 x 10^3 in 3rd, 4th and 5th larval instars of *P. brassicae*, respectively. There were significant differences between the instars of the same insect species with respect to the production of IJs/g of host body weight (Table 4). The less production of entomopathogenic nematodes in 3rd larval instars may probably be due to not only of less food availability but also of completion of less number of generations at the time of food exhaustion. The results of the present studies are in conformity of the observations of Khan *et al*. (2007) who had harvested 79.145 x 10^3 IJs/larva of *P. brassicae*. Rajkumar *et al*. (2002) obtained an average yield of 2, 01,520 IJs/insect for *Heterorhabditis* sp. and 90,945 IJs/insect for *Steinernema* sp. on *Galleria mellonella* larvae sized 23-25 mm long/227 mg weight and 20-22 mm long/223 mg weight, respectively.

The total time period between the larval mortality and the initiation of emergence of the entomopathogenic nematode infective juveniles from the cadavers ranged from 7-8, 9-10 and 10-11 days in case of 3rd, 4th and 5th larval instars of *P. brassicae*, respectively. In all the host cadavers, the initiation of nematode emergence started earlier in 3rd larval instars followed by 4th and 5th larval instars. The total nematode progeny emergence period continued from 17-19, 23-25 and 26-29 days after mortality in case of 3rd, 4th and 5th larval instars of *P. brassicae*, respectively (Table 4). The total duration between larval mortality and the peak period of emergence of entomopathogenic nematode infective juveniles from the cadavers of 3rd, 4th and 5th larval instars of *P. brassicae* ranged from 10-13, 14-19 and 16-21days after mortality, respectively (Table 4). The early emergence of the juveniles in 3rd larval instars may be attributed to the exhaust of food for nematode's growth and reproduction which resulted in exit of nematodes from the cadavers. Contrary to it, the larval mass in 5th instar would have supported the nematode's growth and reproduction with sufficient food material and hence the exit of juveniles from the cadaver would have delayed. 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 cadavers and that the numbers of infective juveniles were also more in larger cadavers than smaller cadavers.

Table 1. Laboratory evaluation of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* against cabbage butterfly, *Pieris brassicae* using Petri dish bioassay technique

Dosage of EPN (IJs/larva)	Per cent mortality of *P. brassicae* over control											
	Post treatment (Hours after exposure)											
	3rd instar larvae				4th instar larvae				5th instar larvae			
	24	48	72	96	24	48	72	96	24	48	72	96
60	63.33 (52.77)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	30.00 (33.21)^a	90.00 (71.56)^a	100.00 (90.00)^a	100.00 (90.00)^a	16.67 (23.85)^a	83.33 (66.14)^a	100.00 (90.00)^a	100.00 (90.00)^a
50	50.00 (45.00)^b	100.00 (90.00)^a	100.0 (90.00)^a	100.00 (90.00)^a	26.67 (30.99)^a	86.67 (68.85)^a	100.00 (90.00)^a	100.00 (90.00)^a	13.33 (21.14)^a	76.67 (61.22)^b	100.00 (90.00)^a	100.00 (90.00)^a
40	43.33 (41.15)^b	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	13.33 (21.14)^b	73.33 (59.00)^{bc}	100.00 (90.00)^a	100.00 (90.00)^a	06.67 (12.29)^b	63.33 (52.78)^c	100.00 (90.00)^a	100.00 (90.00)^a
30	26.67 (30.99)^c	86.67 (68.85)^b	100.00 (90.00)^a	100.00 (90.00)^a	06.67 (12.29)^c	66.67 (54.78)^c	100.00 (90.00)^a	100.00 (90.00)^a	00.00 (00.00)^c	50.00 (45.00)^d	100.00 (90.00)^a	100.00 (90.00)^a
20	20.00 (26.56)^d	73.33 (59.00)^c	100.0 (90.00)^a	100.0 (90.00)^a	00.00 (00.00)^d	53.33 (46.92)^d	93.33 (77.71)^b	100.00 (90.00)^a	00.00 (00.00)^c	46.67 (43.08)^d	86.67 (68.85)^b	100.00 (90.00)^a
10	00.00 (00.00)^e	40.00 (39.23)^d	53.33 (46.92)^b	73.33 (59.00)^b	00.00 (00.00)^d	16.67 (23.85)^e	40.00 (39.23)^c	56.67 (48.85)^b	00.00 (00.00)^c	00.00 (00.00)^e	36.67 (37.22)^c	46.67 (43.08)^b
0	00.00 (00.00)^e	00.00 (00.00)^e	00.00 (00.00)^c	00.00 (00.00)	00.00 (00.00)^d	00.00 (00.00)^e	00.00 (00.00)^c	00.00 (00.00)^c	00.00 (00.00)^c	00.00 (00.00)^e	00.00 (00.00)^d	00.00 (00.00)^c
CD (ρ=0.05)	7.09 (4.40)	5.25 (3.90)	3.90 (2.50)	3.90 (2.58)	7.09 (8.48)	9.24 (6.39)	3.90 (7.16)	3.90 (2.25)	7.09 (8.48)	6.34 (4.27)	5.00 (3.60)	3.90 (2.25)

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

Table 2. Concentration mortality response of *Pieris brassicae* isolate of EPN, *Heterorhabditis bacteriophora*

Larval instar	Parameter (IJs/larva)	Post treatment (Hours after exposure)			
		24	48	72	96
3rd	LC_{50}	46.61	12.53	9.91	9.32
	SD	6.853	7.127	5.715	3.265
4th	LC_{50}	76.33	18.92	11.05	9.82
	SD	3.920	8.091	7.229	5.307
5th	LC_{50}	91.36	29.02	11.73	10.08
	SD	2.228	8.944	7.600	6.532

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

Table 3. Time mortality response of *Pieris brassicae* isolate of EPN, *Heterorhabditis bacteriophora*

Larval instar	Parameter (Hours after exposure)	Post treatment (IJs/larva)					
		10	20	30	40	50	60
3rd	LT_{50}	65.15	34.48	30.51	24.39	23.99	23.12
	SD	9.291	11.313	10.503	8.500	7.500	5.500
4th	LT_{50}	84.54	47.61	39.79	36.32	30.51	29.20
	SD	7.505	13.796	13.205	12.261	10.504	10.099
5th	LT_{50}	105.36	50.24	47.97	40.46	35.68	33.52
	SD	7.320	13.503	14.361	13.225	12.284	11.902

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

Table 4. Recovery of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* from cabbage butterfly, *Pieris brassicae*

Stage of insect larva	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)
		Min.	Max.	Avg.				
3rd	0.08	6.62 (3.82)	7.19 (3.85)	7.02 (3.84)^c	91.49 (4.96)^c	7-8	10-13	17-19
4th	0.17	24.43 (4.39)	26.16 (4.42)	25.74 (4.41)^b	151.30 (5.18)^b	9-10	14-19	23-25
5th	0.25	57.71 (4.76)	59.42 (4.77)	58.45 (4.76)^a	233.78 (5.37)^a	10-11	16-21	26-29
C.D. (P = 0.05)		5.87 (0.08)	5.54 (0.08)	5.39 (0.08)	5.38 (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 DMR



Healthy larvae



EPN, *Heterorhabditis bacteriophora* infected larvae (Cadavers)

Plate 1. Virulence of Kashmir isolate of EPN, *Heterorhabditis bacteriophora* on cabbage butterfly, *Pieris brassicae* in Kashmir

ACKNOWLEDGEMENT

The senior author is highly thankful to Dr. M. Nagesh, Principal Scientist (Nematology) ICAR-NBAIR Bangalore, for identification of the entomopathogenic nematode isolated from Kashmir.

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