Factors affecting propagation of *Heterorhabdittis bacteriophora* Poinar, an entomopathogenic nematode in *Plutella xylostella* (L.)

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ABSTRACT

Entotomopathogenic nematode, *Heterorhabittis bacteriophora* possess tremendous potential for biological control of *Plutella xylostella* (L.) commonly known as the diamond back moth (DBM), major pest of cabbage and cauliflower. Five parameters *viz.*, incubation dose, host body weight, incubation temperature, host food plant and host feeding status were taken to determine their effect on the propagation of *H. bacteriophora* in final instar of *P. xylostella*. The number of infective juveniles (IJs) produced increased with the increase in the inoculation dose up to 15 (IJs) per larva. Further increase in dose adversely affected the nematode progeny production because of overcrowding. A positive correlation between host body weight and number of IJs/mg body weight was observed (r=0.8343). Optima temperature for *H. bacteriophora* was found to be 25ºC and the range was 15ºC to 35ºC. There was no significant difference in nematode progeny production and host food plant viz., cabbage, cauliflower, knolknol and mustard. Similarly IJs production per mg body weight was not significantly different in starved and fed larvae (host feeding status).

Keywords: Propagation, Incubation dose, Host body weight, Incubation temperature, Host food plant, Host feeding status

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INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is one of the most important tropical ornamental bulbous flowering plant cultivated for production of long lasting flower spikes. It is popularly known as Rajanigandha or Nishigandha which means night fragrant.

Plutella xylostella (L.) commonly known as the diamond back moth (DBM) is an abnoxious pest of crucifers (Talekar and Shelton, 1993; Gautam *et al.*, 2018). It has developed resistance to most of the insecticides (Raju and Singh, 1995; Jiang *et al*., 2015 and Meghana *et al*., 2018). In an endeavor to search for an alternative pest management technology, entomopathogenic nematodes have been tested against insect pests (Gaugler and Kaya, 1990; Bhatnagar *et al*., 2004 and Sankaranarayanan and Askary, 2017). Out of eight entomopathogenic nematode species tested against DBM larvae in India, *Heterorhabditis bacteriophora* Poinar was found to be most pathogenic (Shinde and Singh, 2000 and Vashisth *et al*., 2017). Further studies have revealed that except eggs, all other developmental stages of DBM *viz.*, larvae, prepupae, pupae and adults were susceptible to this nematode species (Singh and Shinde, 2002 and Rishi and Prasad, 2012). Present investigations were undertaken to study the effect of various factors viz., incubation dose, host body weight, temperature, host food plant and feeding status (starved/fed) of the host on the propogation of *H. bacteriophora* in final instar DBM larva, with a view to obtain informations, which could be used for effective planning of strategies for the management of *Plutella xylostella*.

Table 1. Effect of inoculation dose on the propagation of *H. bacteriophora* in final instar larvae of diamond moth, *P. xylostella*, IJs= Infective Juveniles

Inoculation dose IJ/Larva	Number of nematodes emerged per larva			Avg. number of IJs/mg body weight	Juveniles (%)	Adults (%)
	Min.	Max.	Avg.			
2	1100	1720	1432[a]	198.9	97	3
5	1310	2040	1674[b]	232.5	99	1
10	1410	2140	1851[ab]	257.1	98	2
15	1580	2340	2006[a]	278.6	99	1
20	1320	2030	1822[ab]	253.1	97	3
25	1390	1910	1650[bc]	229.2	87	13
30	1300	1970	1616[c]	224.4	81	19
CD at 5% 238.44; SEm 82.65; CV% 15.18						

MATERIALS AND METHODS

Plutella xylostella were raised in the laboratory on cauliflower (*Brassica oleracea* var. capitata L.) seedlings. Test nematode *Heterorhabditis bacteriosphora* was multiplied in vivo on greater wax moth, *Galleria mellonella* (Linn.) larvae at 25^{o}C, using the method described by Dutky *et al*. (1964). The infective juveniles (IJs) of the nematode were stored in tissue culture flasks (100 mL capacity) at 10^{o}C before use. Following five parameters *viz.,* incubation dose, host body weight, incubation temperature, host food plant and host feeding status were taken to determine their effect on the propagation of *H. bacteriophora* in final instar of *P. xylostella*.

For inoculation dose parameter, thirty DBM larvae (Avg. body weight 7.2 mg) were exposed to nematode in 50 mm diameter inoculation arena (15 larvae per arena). The inoculation doses included in the test were 2, 5, 10, 15, 20, 25 and 30 IJs/larva. Ten dead were randomly selected and transferred to nematode recovery traps to observe nematode emergence. For host body weight parameter, larvae of four different sizes *viz.*, late third, fourth, fifth and late final instars weighing 2.5, 4.6, 6.2 and 7.8 mg body weight, respectively. For each group, 20 larvae were exposed to 400 IJs in 50 mm diameter inoculation arena.

To evaluate effect of temperature, six batches of 20 DBM larvae (Avg. body weight 7.4mg) were exposed to 400 IJs in 50 mm diameter inoculation arena. And after 48 hours the dead larvae were individually put in nematode recovery traps. The inoculation arenas and nematode recovery traps were held at different temperature viz., 10^{o}, 15^{o}, 20^{o}, 30^{o} and 35^{o}C. At each temperature 10 larvae were taken for recording observations on nematode propagation daily. To evaluate the effect of host food plant, four batches of DBM larvae were reared on four different host plants viz., cabbage, cauliflower, knolknol and mustard throughout their larval period, starting from first instar. The average body weight of final instar larvae taken from cabbage, cauliflower, knolknol and mustard 7.1, 7.4, 6.9 and 6.7 mg, respectively. 20 final instar larvae were exposed to 400 IJs each in 50 mm diameter inoculation arena. After 48 hours, 10 dead larvae were randomly picked and kept in recovery traps for recording nematode emergence. For feeding status parameter, twenty larvae of penultimate instar pre starved for 48 hours were exposed to 400 IJs in 50 mm diameter inoculation arena. 10 dead larvae were transferred to recovery traps and total number of IJs per larva were recorded and compared with similar observations recorded with cabbage fed larva.

In each test final instar DBM larva were exposed to nematode IJs, when the insects died, they were individually transferred to nematode recovery traps which were basically similar to the one initially described by White (1927) and subsequently modified by Woodring and Kaya (1988) and observed daily for emergence of IJs from the cadavers. Total number of IJs emerging from the cadaver on each day were recorded till no further nematode emergence was observed. All the data obtained from above said five parameters

Table 2. Effect of host body weight on the propagation of *H. bacteriophora* in the larvae of diamond moth, *P. xylostella*, IJs= Infective Juveniles

Average weight per larva	Number of nematodes emerged per larva			Avg. number of IJs/mg body weight	Juveniles (%)	Adults (%)
	Min.	Max.	Avg.			
2.5	210	810	482	192.80	65	35
4.6	580	1130	862	187.39	88	12
6.2	1320	1920	1664	268.38	94	6
7.8	1590	2240	1931	285.95	96	4
CD at 5% 198.612, SEm 68.84; CV% 17.63						

were subjected to analysis of variance and probit analysis for host body weight. Correlation between dose, host body weight and host plant respectively with number of IJs harvested per larva per mg weight was calculated.

RESULTS AND DISCUSSION

When *H. bacteriophora* progeny was recorded at inoculation doses ranging from 2 to 30 IJs per larva, an increase in the number of nematodes emerging per larva with the increase in the inoculation dose was observed up to a dose of 15 IJ/larva (r^2 = 0.9698) and with further rise in the dose from 15 IJ to 30 IJ/larva progeny production declined (negative correlation coefficient, r = - 0.9662 (Table 1). Similar observations were reported by Shapiro-IIan and Gaugler (2010) and Selvan *et al.* (1993) that nematode production of *H. bacteriophora* in *G. mellonella* increased with the increase in the dose up to certain extent and then declined. Kondo (1989) also reported that an increase in the inoculation dose of *Steinernema feltiae* resulted in higher emergence of IJs from *Spodoptera litura* larvae. As very narrow range of doses was taken by Kondo (1989), they observed no decline in progeny production with the rise in dose.

Examination of *H. bacteriophora* propagation in DBM larvae of four different weight showed that nematodes were more numerously produced in larger larvae (Table 2). The weight of larvae and the number of nematodes recovered/larva were positively correlated (r^2 = 0.9773). Kondo (1989) also observed delay in *S. feltiae* IJs emergence from larger *S. litura* larvae and the number of nematodes/larva was more with larger larvae. Similarly, when the efficacy of three entomopathogenic nematodes (EPNs) *viz.*, *S. feltiae, S. carpocapsae* and *H. bacteriophora* was evaluated against larvae of *T. absoluta* by Damme *et al*. (2015) it was found that all EPNs were effective to all four larval instars of *T. absoluta* but caused higher mortality in the later instars than in the first instars.

Table 3. Effect of temperature on the propagation of *H. bacteriophora* in the larvae of diamond back moth, *P. xylostella*, IJs= Infective Juvenile

Temperature (°C)	Number of nematodes emerged per larva			Avg. number of IJs/mg body weight	Juveniles (%)	Adults (%)
	Min.	Max.	Avg.			
10	-	-	-	-	-	-
15	610	1120	880	118.92	97	3
20	820	2730	2219	315.93	96	4
25	1430	2240	1805	243.92	97	3
30	910	1520	1134	153.24	96	4
35	-	-	-	-	-	-
CD at 5% 253.59; SEm 87.39; CV% 18.31						

When DBM larvae infected with *H. bacteriophora* were incubated at different temperature, nematode was found to grow and multiply in the temperature range of 15°C to 35°C with maximum number of nematodes produced at 25°C (Table 3). At temperatures below and above 20°C, decline in the nematode production was recorded. Present study is in confirmation with findings of Grewal *et al.* (1994) on *G. mellonella* who

Table 4. Effect of host food plant on the propagation of *H. bacteriophora* in the final instar larvae of diamond back moth, *P. xylostella,* IJs= Infective juveniles

Host food plant	Number of nematodes emerged per larva			Avg. number of IJs/mg body weight	Juveniles (%)	Adults (%)
	Min.	Max.	Avg.			
Cauliflower	1430	2510	1985	279.58	97	3
Cabbage	1320	2410	1934	261.35	98	2
Kholkhol	1390	2270	1835	265.94	96	4
Mustard	1410	2120	1727	257.76	97	3

reported 20^{o}C as temperature optima for nematode species/strains have well defined thermal niche and temperature optima for propagation. Similar results were reported by Damme *et al*. (2015) where *S. carpocapsae* and *H. bacteriophora* performed better at 25°C than at 18°C, while *S. feltiae* caused 100% mortality at both temperatures. A higher efficacy of *S. carpocapsae* and *H. bacteriophora* at 25^{o}C than at 18^{o}C has also been reported before by Kamali *et al*. (2013) and Lacey *et al*. (2005).

When larvae reared on four different host plants *viz.,* cabbage, cauliflower, knolknol and mustard, were inoculated with *H. bacteriophora*, no significant difference was observed in the number of nematodes produced in the larvae reared on different host plants (Table 4) which is supported by Miranda *et al*. (2013), who reported that nutritional status did not significantly affect time of emergence of IJ progeny. Unlike the present results, Barbercheck *et al*. (2003), Agrawal (2005) and Hazir *et al*. (2016) concluded that EPNs response were dependent on the nematode isolate and the particular host plant on which the insect feeds. Barbercheck (1993) reported *S. carpocapsae* and *H. bacteriophora* progeny production from carrot root worm, *Diabrocita undecimpunctata howardi* that had been fed on squash root, was significantly lower than the nematode progeny production from root worms that had been fed on corn roots. Barbercheck in this experiment had included plants belonging to different families which were expected to vary greatly in their chemical composition nutritional value, but in present study, the host plants were from same family (cruciferae) and thus no significant difference in growth and development was observed in larvae fed on four different host plants. Epsky and Capinera (1994) also found that *S. carpocapsae* progeny production was lower in collard fed larvae of *Agrotis ipsilon* than in larvae fed on artificial diet. When Singrin (found in collard) was added to the artificial diet, suppression in nematode progeny production was observed. Thus Singrin act as inhibitor for the nematode development which is also present in all the four plants included in the present study as reported by Kjaer (1960) and Ettlinger and Kjaer (1968). This explains why host plants were not found to affect nematode progeny production in the present study.

Starved final instar of DBM larvae were found to produce less number of nematodes/larva as compared to well fed larvae. However the number of IJs/mg host body weight was almost equal in fed and starved larvae. Average number of nematodes recovered from single larva were 1934 with larvae fed on cabbage weighing 7.4mg (Table 4) as against 1720 recovered from starved larvae weighing 6.4 mg. but number of IJs harvested/mg host body weight were 261.35 for cabbage fed larvae and 268 for starved larvae which was statistically not significant. Therefore no effect of host starvation on nematode propagation was seen except that due to less body weight and the total number of IJs harvested were less but the number of IJs host body weight were not affected by starvation. Parallel observations were recorded by Kondo (1989) with a different species of nematode and insect describing the difference in number of progeny produced/larva was probably due to differences in their weight only.

Through present investigation basic information on the host pathogen relationship has been generated which is essential for exploring the possibilities of using this

nematode species of the management, DBM under vegetable cropping system.

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