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SUITABILITY OF BLACK SOLDIER FLY LARVAE AS HOST FOR ENTOMOPATHOGENIC NEMATODES

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ABSTRACT

Black soldier fly *Hermetia illucens* (L.) larvae can serve many uses, of which potential exists for its use as a host for mass production of entomopathogenic nematodes. This study evaluates its larval instars for their suitability for rearing of *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser). The results revealed that 4th instar larvae is economical and effective, and this can be used at farm level. Thus, it can be popularized for mass production of entomopathogenic nematodes. The nematodes/ cadaver was found to be half as that from *Galleria*, and hence two 4th instar cadavers can be recommended in place of a single *Galleria* cadaver. The results also showed that *H. illucens* is unsuitable for mass multiplication of *S. carpocapsae*.

Key words: *Hermetia illucens*, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Galleria mellonella*, insect rearing, mass production, larval instars, cadavers

Black soldier fly *Hermetia illucens* (L.) (Stratiomyidae: Diptera) is a beneficial insect as it can be used for waste management with minimum carbon footprint, besides its other uses. It can be easily reared in any organic waste material and its biology had been studied by Sharanabasappa et al. (2019), and with biological parameters compared on different food wastes (Srikanth and Deshmukh, 2021). The present study assessed the use of *H. illucens* larva as host for the rearing of entomopathogenic nematodes. These nematodes (EPN) are being advocated as the safest biopesticides. Entomopathogenic nematodes of genera *Steinernema* and *Heterorhabditis* are efficient biocontrol agents (Koppenhofer, 2000). Nematode infected cadavers of greater wax moth (*Galleria mellonella* L.) larvae are the popular ones for the rearing of these nematodes, but their rearing and maintenance requires sufficient expertise and care. This study evaluates the suitability of *H. illucens* larvae as hosts for cost effective mass multiplication of some entomopathogenic nematodes.

MATERIALS AND METHODS

The experiment was conducted at the Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala (N8^{0}25'37.13952", E76^{0}59'12.50736", 29 masl) under laboratory condition during 2020-21. Egg masses of *H. illucens* were collected in cardboard honeycombs from a compost bin set up for attracting the naturally occurring females for oviposition (Ewusie et al., 2019). These were transferred to plastic pots (17 and 25 cm dia, 30cm high) containing vegetable and fruit waste. Suitable larval instars were collected from these and simultaneously reared final instar larvae of *G. mellonella* on standard semisynthetic diet were used as check. Entomopathogenic nematodes viz., *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser), obtained from the Banana Research Station, Kannara and ICAR-NBAIR Bengaluru, respectively were used. Nematodes were maintained in the laboratory (28±2^{0}C, 60-70%RH) as aqueous suspension as well as infected cadavers. There were 10 treatments (T1: 5th instar BSFL+ *H. bacteriophora*, T2: 4th instar BSFL +*H. bacteriophora*, T3: 3rd instar BSFL + *H. bacteriophora*, T4: *Galleria* larva+ *H. bacteriophora*, T5: 5th instar BSFL+ *S. carpocapsae*, T6: 4th instar BSFL +*S. carpocapsae*, T7: 3rd instar BSFL + *S. carpocapsae*, T8: *Galleria* larva+ *S. carpocapsae*, T9: 6th instar BSFL + *H. bacteriophora* and T10: 6th instar BSFL + *S. carpocapsae*) replicated four times under completely randomized design. One

replication consisted of 10 uniform sized larvae of respective instars in a 90 mm petriplate lined with Whatman number 1 filter paper disc. The larvae were exposed to 100 infective juveniles (IJs) of respective nematodes per larva (Koppenhofer and Kaya 1998).

For this purpose, freshly emerged IJs were collected from Whites trap (White, 1927) in double distilled water and diluted to obtain the required number of IJs; the solution was applied to filter paper disc in petri plate. A waiting period of 10 min was given to ensure uniform distribution of IJs and the uniform soaking of filter paper, larvae were introduced and closed. The petriplates were incubated at laboratory conditions at room temperature. Observations were taken at an interval of 24 hr on mortality. Absolute control was set using double distilled water with above mentioned insects and was also replicated four times. Half of the cadavers formed in various treatments were kept in petri plates in laboratory conditions under room temperature and observed daily for colour change, intactness of cadaver and natural emergence of nematodes. Other half of the cadavers were kept in White trap and number of IJs emerged were observed and counted under stereo zoom microscope on 24 hr basis. Viability of IJs from BSF larvae were found out by infecting them against *Galleria* larvae. The data obtained were subjected to statistical analysis using the WASP-Web Agri Stat Package 2.0 programme developed by ICAR- Central Coastal Agricultural Research Institute, Goa.

RESULTS AND DISCUSSION

The results revealed that treatments involving only double distilled water did not cause mortality (these not shown in tables); all larvae were alive in the treatments T5: 5th instar BSF + *S. carpocapsae*; T9: 6th instar BSF + *H. bacteriophora* and T10: 6th instar BSF + *S. carpocapsae*; and larvae in these successfully completed their lifecycle. Mortality of larvae when observed daily for 10 days, it was seen that after 24 hr, mortality was there only in *Galleria* larvae (Table 1). The EPN, *H. bacteriophora* was able to bring about mortality among *H. illucens* larval instars from second day; and all larvae in T4: *Galleria* larva+ *H. bacteriophora* also died on 2 DAI. On the third day, *S carpocapsae* could bring about 100% mortality of *Galleria* larvae (T8), whereas 100% mortality was observed in T3 (3rdinstar BSF + *H. bacteriophora*) after 5 days. *Galleria mellonella*, the universal host of entomopathogenic nematodes

Table 1. Mortality of BSF larvae and *Galleria* larvae treated with entomopathogenic nematodes

Treat-	Mortality at different days interval (%)										Total
ments	1	2	3	4	5	6	7	8	9	10	
	0	2.5	2.5	5	5	5	5	5	5	5	5
T1	(0.91)c	(5.29)e	(5.29)d	(9.67)de	(9.67)de	(9.67)de	(9.67)d	(9.67)d	(9.67)d	(9.67)d	(9.67)e
	0	37.5	75	80	85	85	85	85	85	85	87.5
T2	(0.91)c	(37.51)d	(61.23)b	(64.18)b	(67.50)b	(67.50)b	(67.50)b	(67.50)b	(67.50)b	(67.50)b	(69.53)b
	0	82.5	82.5	97.5	100	100	100	100	100	100	100
T3	(0.91)c	(65.47)c	(65.50)b	(84.71)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a
	85	100	100	100	100	100	100	100	100	100	100
T4	(67.87)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a
	0	0	0	0	0	0	0	0	0	0	0
T5	(0.91)c	(0.91)e	(0.91)d	(0.91)e	(0.91)e	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)f
	0	0	0	7.5	17.5	17.5	40	45	45	45	35
T6	(0.91)c	(0.91)e	(0.91)d	(11.70)d	(18.34)d	(18.34)d	(38.95)c	(42.12)c	(42.12)c	(42.12)c	(36.22)d
	0	2.5	7.5	32.5	42.5	57.5	67.5	75	80	82.5	77.5
T7	(0.91)c	(5.29)e	(14.05)c	(34.50)c	(40.39)c	(50.14)c	(59.00)b	(63.52)b	(66.53)b	(68.19)b	(61.77)c
	75	92.5	100	100	100	100	100	100	100	100	100
T8	(60.64)b	(78.30)b	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a	(89.09)a
	0	0	0	0	0	0	0	0	0	0	0
T9	(0.91)c	(0.91)e	(0.91)d	(0.91)e	(0.91)e	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)f
	0	0	0	0	0	0	0	0	0	0	0
T10	(0.91)c	(0.91)e	(0.91)d	(0.91)e	(0.91)e	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)d	(0.91)f
CV	27	22.15	17.67	17.91	21.54	22.1	19.16	16.45	14.95	14.37	8.43
CD (0.05)	5.29	9.10	8.37	9.98	12.62	13.27	12.31	10.75	9.84	9.49	5.44
SEM	3.36	9.93	8.39	11.93	19.10	21.10	18.17	13.87	11.61	10.81	1.90

T1: 5th instar BSF+ *H. bacteriophora*, T2: 4th instar BSF +*H. bacteriophora*, T3: 3rdinstar BSF + *H. bacteriophora*, T4: *Galleria* larva+ *H. bacteriophora*, T5: 5th instar BSF + *S. carpocapsae*, T6: 4th instar BSF +*S. carpocapsae*, T7: 3rdinstar BSF + *S. carpocapsae*, T8: *Galleria* larva+ *S. carpocapsae*, T9: 6th instar BSF + *H. bacteriophora* and T10: 6th instar BSF + *S. carpocapsae;* * Values in parentheses after arc sine transformation

proved its superiority. Alonso et al. (2018) found that *S. carpocapsae* activation was much higher to insect homogenates of *G. mellonella* than to *H. illucens*. This is slightly in disagreement with the findings of Toutoris et al. (2017) who observed that fourth instar of *H illucens* was the most susceptible, and third instar was more prone to speedy nematode infections since both nematodes caused mortality within 48 hr. Later instars of *H. illucens* are known for their tough integument due to deposition of calcium carbonate (Johannsen, 1922); their larvae have only two pairs of functional spiracles and the integument is shagreened due to deposition of calcium carbonate (Stehr, 2008).

Comparing the nematodes, *H. bacteriophora* gave quick mortality than *S. carpocapsae;* the former not only utilized natural openings but it got attached to host cuticle and entered with a drill like mechanism and caused changes in host behavior and produced septicemia within 6 hr in *Drosophila* maggots (Dziedziech et al., 2020). The results clearly indicated that mortality decreased with age of the larvae. A similar trend was also observed by Tourtois et al. (2017). Younger larvae of Mexican fruit fly *Anastrepha ludens* (Loew) were found more susceptible to *H. bacteriophora* (Toledo et al., 2005) than older ones; which is in agreement with the present findings. Susceptibility of younger larvae to EPN was also demonstrated by Fuxa et al. (1988) in the case of *S. feltiae* exposure to fall armyworm larvae.

Number of infective juveniles of EPN that emerged from cadavers were a direct indicator of host suitability for mass multiplication. From the *H. bacteriophora* infected *Galleria* cadaver, 4.51 lakhs of IJs emerged, whereas from 4th instar of *H. illucens*, 2.3 lakhs *H. bacteriophora* IJs were seen emerging. Rahoo et al. (2019) also observed >4.5 lakhs *H. bacteriophora* IJs emerging from a single final instar *G. mellonella* cadaver. The present observations in this respect are not in agreement with that of Tourtois et al. (2017) who observed tenfold less IJs of *H. bacteriophora* from *H. illucens* than from *G. mellonella*, even after injuring the larval cuticle. *Hermetia illucens* BSF was observed not at all suitable for *S. carpocapsae* mass production; though 4th instar larva produced 1.1 lakh IJs; the emergence was not uniform in all replications. Increased infection rate and mortality did not lead to an increase in the number of IJs harvested from *H. illucens* exposed to *Steinernema* spp. (Tourtois, 2014). In the case of *H. bacteriophora*, IJs started emerging from *G. mellonella* and 4th instar of *H. illucens* from the 6th day

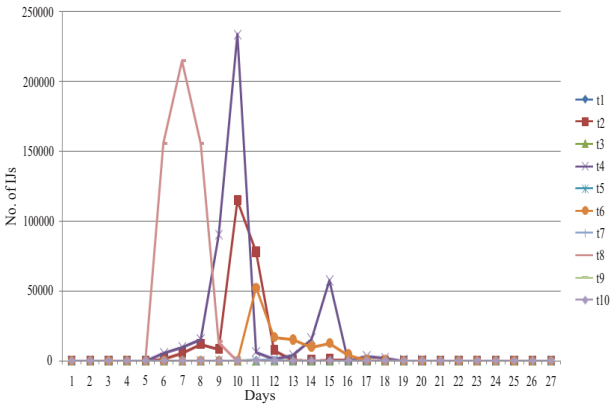
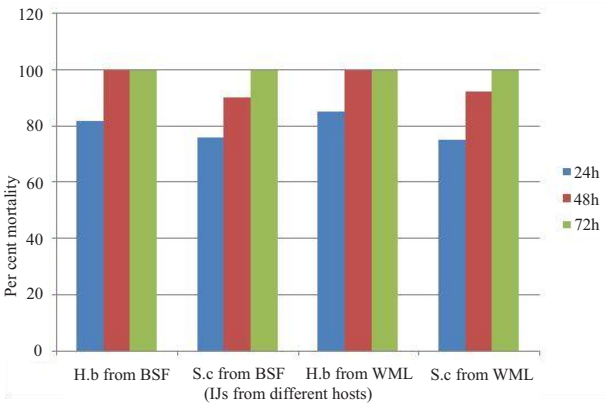


Fig. 1. Daily emergence of infective juveniles from cadavers

but cease to emerge from 27 and 23rd day, respectively (Fig. 1). Peak emergence of *H. bacteriophora* was seen on the 10th day on both of these cadavers. A similar trend was observed by Rahoo et al. (2019) who observed a peak on the 13th day in the case of *H. bacteriophora* from *Galleria* cadavers. For *S. carpocapsae*, infective juveniles emerged from 6th to 16th day, 11th to 21st day and 11th to 13th day in *Galleria*, third and fourth instar *H. illucens* larvae, respectively; though third instar was more susceptible, these produced less nematodes/ insect.

Cadavers formed by *H. bacteriophora* were initially colourless, turning pale yellowish orange within a day, then to brick red and chocolate brown within 4 to 6 days. All cadavers were flaccid initially, but they were seen gaining turgidity slowly within 5 to 7 days. Cadavers formed due to attack of *S. carpocapsae* were initially colourless, flaccid and softer than that of *H. bacteriophora*, turned to pale grey and to dark grey in *Galleria*; while *H. illucens* cadavers also shown variations in colour from colourless to various shades of grey, but the colour did not get intensified. Natural egress of infective juveniles was observed in the case of cadavers of *G. mellonella* (both nematodes) and *H. bacteriophora* infected 4th instar *H. illucens* only. Nematodes were seen emerging from the mouth in the case of *H. illucens* and through all natural openings in *G. mellonella* initially. Infective juveniles of both species of nematodes from *H. illucens* cadavers were found viable since 100% mortality was observed in *Galleria* larvae when inoculated with these IJs emerged from *H. illucens* BSF (Fig. 2). This clearly indicated that EPN emerged from *H. illucens* are as viable and effective as that from *G.mellonella*. Thus, it can be concluded that fourth instar *H. illucens* can be utilized for the mass production of *H. bacteriophora*.



(H.b: *H. bacteriophora*, S.c: *S. carpocapsae*, WML: wax moth larva)

Fig. 2. Mortality of *G. mellonella* larvae by IJs from two hosts

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AUTHOR CONTRIBUTION STATEMENT

MCR, NA and GR conceived research. GR helped in designing the research and provided guidance in carrying out nematological techniques and provided nematode culture. PYPI contributed to statistical analysis. MCR prepared the draft manuscript. All authors reviewed, updated and finalized the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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