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Virulence study of Native entomopathogenic nematodes (4 strains) and mass production of EPN by using different soild media

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Abstract
Entomopathogenic nematodes from families steinernematidae and Heterorhabditidae are the important natural enemies of insects. *In vivo* and *in vitro* production technology is a key factor in the success of implementing EPNs as a biological control agent. In the present study, 4 strains of Entomopathogenic nematodes belong to the genus *steinernema* (2), *Heterorhabditis* (2) were assessed against *Corcyra cephalonica* and also demonstrate *in vivo* and *in vitro* techniques that are commonly used for reproduction of nematodes. These EPN included *Heterorhabditis indica, H. bacteriophora, Steinernema glaseri, S. siamkayai*. Virulence of all these strains was tested against insect pests using bioassay method. After *in vivo* and *in vitro* production (10 media) of nematode inoculum, different parameters for optimizing infective juveniles recovery, yield were investigated. Among all these media, *H. bacteriophora* and *H. indica*, produced more no of infective juveniles in NA medium-I. For *S. siamkayai* and *S. glaseri*, reproductivity is more in Modified dog biscuit medium. As comparing these parameters, *S. glaseri* and *H. indica* were highly virulent than *H. bacteriophora* and *S. siamkayai*. In comparative study between *in vivo* and *in vitro* production, the best one was *in vivo* method and reproductivity is also high compared to *in vitro* method.

Keywords: Entomopathogenic nematodes, *Steinernema spp*., *Heterorhabditis spp*., *In vivo* and *In vitro* production

1. Introduction
Entomopathogenic Nematodes were reported with biological and economic importance in the control of pests, especifically of the orders Coleoptera, Dictyoptera, Lepidoptera, Hemiptera, and Orthoptera. The only free- living stage, known as the infective juvenile (IJ) or dauer stage, penetrated the body of insect larvae, they release mutualistic bacteria symbionts that kill the host within 48–72 h; After that, they develop, reproduce, and complete its cycle with one to three generations depending on the size of the host (Georgis and Gaugler 1991; Laznik and Trdan 2014) [16, 35]. The EPNs are found naturally in the soil environment and contribute to the insects' control (Poinar, 1979) [21]. There is a relationship of EPN with bacteria, where each species of nematode is tightly associated with a certain species or subspecies of members Enterobacteriaceae family. This apparently symbiotic relationship between the nematode and its bacteria has been considered the main factor responsible for the death of the insect (Poinar, 1979) [21]. Commercially produced EPNs are currently in use for control of scarab larvae in lawns and turf, fungus gnats in mushroom production, invasive mole crickets in lawn and turf, black vine weevil in nursery plants and Diaprepes root weevil in citrus, *Corcyra cephalonica*, *Galleria mellonella* in addition to other pest insects. Hence the research work is aimed to prospect and characterize the native EPN.
Entomopathogenic nematodes are symbiotically associated with bacteria of Xenorhabdus / Photorhabdus (Enteriobacteriaceae). The symbionts also produce antifungal and antibacterial metabolites like xenorhabdin, xenocoumacins, nematophines, xerodes and solubile proteinous compounds; hence entomopathogenic nematodes are broad spectrum bioagents for integrated pest and disease management (Seenivasan *et al*. 2012) [23].
Some entomopathogenic nematodes, "cruisers", actively move through the soil to locate an insect host. They work better against insects that are generally less active and that are found beneath the soil surface (Campbell and Gaugler, 1997; Seenivasan and Sivakumar, 2014) [6, 24]. Other entomopathogenic nematodes, "ambushers", tend to remain near the soil surface and

attach to and infect mobile hosts at the soil-litter interface, are more effective against insects that actively move over the soil. (Campell and Gaugler, 1993; Nagachandrabose and Baidoo, 2018) [26] Entomopathogenic nematodes are currently produced by different methods either *in vivo* or *in vitro* (solid and liquid culture) (Seenivasan, 2017) [25]. The most important requirement for successful and economically reasonable usage of EPNs in crop protection is their production on large scale at competitive cost within a short time (Ehlers, 2001) [11]. Hence, this study compared mass production and virulence of EPN reproduced on *in vivo* and *in vitro* production methods, compared their virulence.

Materials and Methods

Mass production of entomopathogenic nematodes

The Four species of EPN isolated for DST-SERB-TARE project were collected from the Sugarcane Breeding Institute, Coimbatore and multiplied on host insects. Therefore, larvae of Rice meal moth, *Corcyra cephalonica* and *Galleria melonella* were used for *in vivo* and *in vitro* production.

Culture of *corcyra cephalonica*

Rice meal moth, *Corcyra cephalonica* was reared on bajra grains. Bajra grains (2½ kg) and 200gms of Groundnut Kernels were broken and filled in 30cm×20 cm plastic trays and eggs of *C. cephalonica* (1 cc/ tray) inoculated into the trays and covered with a muslin cloth. Fully grown larvae of *C. cephalonica* were collected after 30 days of inoculation and used for further studies. Weights of live and dead larvae were also noted. Generally, nematode yield is proportional to host size (Blinova and Ivanova, 1987; Flanders *et al*., 1996) [4, 13]. Yet yield per mg insect (within host species) and susceptibility to infection is often inversely proportional to host size or age (Dutky *et al*. 1964; Blinova and Ivanova 1987; Dolinski *et al*., 2007; Dias *et al*., 2008) [10, 4, 8, 7].

Infectivity - Maintenance of Mother culture

The insect larvae were exposed to the nematode as per the method described by Bedding (1984) [2]. Ten final instar larvae of *C. cephalonica* were released over two layers of Whatmann No. 1 filter paper on 9cm dia Petri dish and the test nematodes were inoculated at a dose of 1 ml of suspensions containing 150 infective juveniles per larva. The Petri dishes were covered with lid and sealed with cling film to conserve moisture. After 48 hrs of inoculation, the dead larvae were counted.

Reproductivity

Heterorhabditis bacteriophora, Heterorhabditis indica, Steirnema. siamkayai and Steinernema glaseri infected *C. cephalonica* larvae were transferred to White's trap (White, 1927) [3] and modified White's trap (plaster of paris) (Woodring and Kaya, 1988) for the emergence of infective juveniles respectively to confirm the reproductivity of the nematodes. These juveniles were washed and rinsed several times with sterile distilled water and stored in a BOD incubator at 20±1^{O}C for *H. indica, H. bacteriophora* and in a refrigerator at 10°C for *S. glaseri, S. siamkayai* in 500ml conical flask until use. The nematode cultures were aerated and changed to fresh sterile distilled water at weekly intervals. The infective juveniles of the entomopathogenic nematodes, *viz*., *heterorhabditids* and *steinernematids,* collected were washed well with 0. 05% formaldehyde and stored in sterile distilled water in conical/tissue culture flasks in BOD respectively.

Virulence of entomopathogenic nematode

In vivo production yields are also dependent on nematode doses (Boff *et al*., 2000) [5]. *Heterorhabditis indica, H. bacteriophora, S. siamkayai and S. glaseri* were selected for testing virulence against *Corcyra cephalonica.* This tests were conducted in 9 cm diameter Petri dishes lined at the bottom with a Whatman No. 1 filter paper and moistened with 1ml sterile distilled water. Infective juveniles were evenly applied over the filter paper. The dosages used were 0, 5, 10, 20,30,35,40 infective juveniles per larva, with 10 larvae per insect per replicate and three replicates for each level. In general nematode yield is proportional to insect host size (Ehlers and Shapiro Ilan, 2005) [12]

The parameters taken were given as follows:

i). Percent mortality of the insects ii). Time taken from exposure to 50% and 100% kill of test insects iii). Yield of infective juveniles per larva iv). Weight of fresh larvae (10 test insects) v). Weight of dead larvae (10 test insects)

In vitro mass culture

In vitro culturing of 4 strains of EPN was tried on 10 different media such as Modified Dog biscuit medium: Dog biscuit - 100g Peptone - 2. 5 g Yeast extract - 5g Beef extract - 25g Groundnut oil - 35ml Distilled water - 500ml. Wout's medium (Wouts, W. M. 1981): Nutrient- broth - 0. 88g Yeast-extract - 0. 32 g Groundnut oil - 10. 40 g Soya flour - 14. 40 g Distilled water - 60 ml. Modified Egg yolk medium: Solid egg yolk - 7. 00g Soya flour - 20. 00g Yeast extract - 2. 00g Sodium chloride - 0. 80g Groundnut oil - 15. 00g Distilled water - 60ml Nutrient agar medium I - beef extract 0. 3 g, peptone 0. 5, g, agar 0. 2 g, NaCl 0. 8 g and chicken fat 10 g; Nutrient agar medium II - beef extract 0. 3 g, peptone 0. 5 g, agar 0. 2 g, NaCl 0. 8 g and soybean oil 5 g; Wheat flour medium - wheat flour 15. 0 g, kabuligram flour 5 g, beef extract 5 g, yeast extract 6 g, agar 1 g and coconut oil 6. 0 g; Modified wheat flour medium - wheat flour 15. 0 g, soyflour 5. 0 g, beef extract 5. 0 g, yeast extract 1 g, and groundnut oil 10. 0 g; Bengal gram medium I - nutrient broth 1. 5 g; yeast extract 0. 7 g, bengal gram flour 20 g and ground nut oil 20 g ; Chicken offal medium- Fresh chicken offal (gall bladders and gizzards removed),Yeast extract. Meat extract media-Chicken offal, Mutton extract powder, Agar, Yeast extract broth, Coconut oil.

The ingredients were mixed together in different composition with polyether polyurethane sponge (1. 5 cm3). The conical flasks were filled with medium mixture (1. 5 g of foam chips: 10 g of medium, w/w) and plugged tightly with cotton. The flasks were autoclaved for 20 minutes at 121°C and allowed to cool at room temperature before inoculation with infective juveniles fresh. The infective juvenile fresh are extracted from the infected insect cadavers and used. The nematodes were inoculated aseptically @ 1000 infective juveniles/flask. Care was taken by avoiding the agitation of flasks after the inoculation of nematodes. The sealed flasks were incubated at 28°C for 30 days. Colonies of the nematodes were observed. The harvesting of the nematodes was done after 30 days. The nematode yield from each treatment medium harvested were expressed in terms of number of infective juveniles/flask (Sunanda and Siddiqui 2013) [29].

Nematode extraction

Nematodes from different media were extracted by replication wise after four weeks of inoculation (Bedding, 1984) [2]. The foam pieces from the flasks were transferred into a tissue paper spread over a 20 mesh aluminium wire mesh. The flasks were thoroughly washed and the washings were also added to the foam pieces. The wire mesh support was then kept in a Petri dish filled with water so that a film of water touches the material over the filter paper. The nematodes were collected from the petri dish and transferred to a one lit. beaker. The nematode suspensions with bacterial cells were allowed to settle and the supernatant was decanted. This process was repeated several times until a clear suspension was obtained. The nematode population was recovered from each suspension over a period of 72 h and pooled together. These nematode populations were further used in laboratory.
Solid culture was first accomplished in two dimensional areas eg. petri dishes, using various media (Hara *et al*., 1981) [18] The media used are Modified Dog biscuit medium, wout's medium, Modified egg yolk medium. House *et al*. (1965) [19] devised a dog food based medium to produce the DD-136 strain of *Neoaplectana carpocapsae* on a commercial scale.
Yield is based on a species or strain basis (Dunphy and Webster, 1989) [9].

Inoculation of bacteria culture

Primary forms of bacterial culture was isolated from *H. indica* and *S. glaseri* respectively, as described by Ansari *et al*. (2003) [1], and cultured on NBTA medium. A loopful of 48 hrs old bacterial culture was inoculated into 250 ml flask containing media and shaken for 24 hrs. at 28° c in dark. Subsequently, the bacterial culture was centrifuged at 4000 rpm for 4 mins.

Statistical Analysis

The data from pathogenicity tests were subjected to Probit analysis (Finney, 1971) [14] for median lethal concentration (LC_{50}) and median lethal time (LT_{50}).

Results and Discussion

Reproductivity

Both *H. indica* and *S. glaseri* were tested for their infectivity caused highest mortality (100 percent) on *C. cephalonica*. But, *H. bacteriophora* and *S. siamkayai* caused only 90 percent infectivity. (Table. 1). For *H. indica*, the highest mortality (100%) was attained when the infective juveniles are applied at the concentrations of 40IJS (36 hrs). For *H. bacteriophora*, the highest mortality (97. 2) was attained at the concentrations of 40 IJS (48 hrs). Sivakumar *et al.* (1988) have obtained 17,108 ± 856 IJs of *H. bacteriophora* from a full grown larva of *C. cephalonica* when inoculated @ 20 IJs/larva in Tamil Nadu.

Table 1: Percentage mortality of insects by *S. glaseri, S. siamkayai, H. indica, H. bacteriophora*

S. NO	Percentage Mortality (%)				Reproductivity
Treatment (IJS)	S. glaseri (36 hrs)	S. siamkayai (72 hrs)	H. indica (36 hrs)	H. bacteriophora (48 hrs)	
T1-0	-	-	-	-	+
T2-5	42. 8	33. 3	36. 6	43. 3	+
T3-10	44. 8	45. 7	46. 7	64. 2	+
T4-20	74	64. 5	53. 3	79. 3	+
T5-30	86. 6	80. 5	80	89. 6	+
T6-35	100	87. 2	95. 5	93. 1	+
T7-40	-	94. 2	100	97. 2	+

(+) –Reproductivity observed

For *S. glaseri*, the highest mortality (100%) was attained at the concentrations of 35 IJS (36 hrs). This study shows that,virulence of *S. glaseri* was high as reported by Banu *et al*. (2004) [17]. They reported that the highest nematode multiplication was noticed in *Steinernema sp.* (12. 01 x 10^3 IJs/weevil) followed by *H. indica* (8. 99 x 103 IJs/weevil) and *S. glaseri* (2. 4 x 10^3 IJs/weevil). For *S. siamkayai*, the highest mortality (94. 2) was attained at the concentrations of 40 IJS (72 hrs). Rajkumar *et al*. (2001) [22] obtained an average yield of 2, 01,520 IJs/insect for *Heterorhabditis sp*. and 90,945 IJs/insect for *Steinernema sp*. on *G. mellonella* larvae sized 23-25 mm long/227 mg weight and 20-22 mm long/223 mg weight, respectively. The results obtained from the experiment coincide with the results of Subramanian (2003) [28]. They revealed that the optimum inoculum level of *H. indica* and *S. glaseri* that would yield the highest level of infective juveniles by *in vivo* method. The mortality percentages was ranges from *S. glaseri* (100%), *H. indica* (100%), *H. bacteriophora* (97. 2), *S. siamkayai* (94. 2). The increased mortality of *Corcyra* caused was concentration dependent.

Median Lethal Concentration (LC_{50})

The *S. glaseri* was found to be highly virulent with an LC50 value of 6. 09 followed by *H. indica* with 7. 01, *H. bacteriophora* with 7. 79, *S. siamkayai* with 8. 05 (Table 2)

Median Lethal Time (LT_{50})

The LT50 of infective juveniles was ranged from 22. 43 to 38. 32hr/larva. The 50 percent larval mortality was taken from 22. 43 h for *S. glaseri*, 27. 88 h for *H. indica*, 32. 41 h for *H. bacteriophora*, 38. 32 h for *S. siamkayai.* The overall results showed that *S. glaseri* and *H. indica* were highly virulent after 36 hrs of inoculation against *Corcyra cephalonica* and reproductivity also attained in a higher manner as compared with *H. bacteriophora* and *S. siamkayai*.

Table 2: Median lethal concentration (LC_{50}) of *S. glaseri, S. siamkayai, H. indica, H. bacteriophora* to *Corcyra cephalonica*

S. NO	Nematode Culture	IJS/Larva	B Value	CHI Aquare Value	Fiducial Limit	
					Lower	Upper
LC_{50}	*S. glaseri*	6. 09	1. 65	0. 27	5. 05	9. 32
	S. siamkayai	8. 05	2. 01	2. 25	6. 16	11. 01
	H. indica	7. 01	1. 88	0. 85	4. 97	9. 47
	H. bacteriophora	7. 79	1. 90	1. 09	6. 26	9. 25
LT_{50}	*S. glaseri*	22. 43	2. 35	4. 01	18. 78	26. 97
	S. siamkayai	38. 32	4. 56	7. 52	33. 14	43. 12
	H. indica	27. 83	3. 25	5. 79	24. 46	31. 33
	H. bacteriophora	32. 41	3. 45	6. 49	20. 22	27. 31

In vitro production of Entomopathogenic nematodes

The *S. glaseri* were multilplied on 7 media out of 10 tried. 100% mortality were attained on Egg yolk-I, Modified dog biscuit medium-I and NA medium-I. The infective juveniles produced from NA medium II caused 97. 2% mortality followed by 95. 5% mortality in Wout's medium. In Bengal gram and modified wheat flour medium, least mortality of 84. 2 and 80. 7 were attained. This study showed that *S. glaseri* multiplied on different media. Vyas *et al*. (1999) [31] reported that *S. glaseri* is produced more on media having fresh chicken extract supplemented with beef and meet extracts on polyurethane sponges. With this technique an average of 50,000 IJs of *S. glaseri* were produced from 1. 5 cm3 polyurethane sponges incorporated with 5 ml medium.

The *H. bacteriophora* multiplied on 8 media out of 10 tried. 100% mortality were attained on 2 media *viz*., Egg yolk-I and NA media. These media produced significantly higher number of infective juveniles than other media. 94. 5% and 94. 3% mortality was attained on Wout's media and NA media-II. Modified dog biscuit, chicken offal medium, Bengal gram medium, Modified wheat flour medium attained significantly least production of 69. 2 – 80. 2. (Table 3)

Table 3: Multiplication of *S. glaseri*, *H. bacteriophora, S. siamkayai and H. indica* on solid media

Test media	Infective juveniles released per 250 ml flask		Mortality of insects	
	S. glaseri	***H. bacteriophora***	***S. glaseri***	***H. bacteriophora***
Wout's medium	25. 61c	27. 89b	95. 5a	94. 5a
Egg yolk-I	35. 54b	37. 02b	100a	100a
NA medium- I	47. 63a	37. 28b	100a	100a
Modified Dog biscuit medium	50. 35a	23. 54c	100a	80. 2b
Wheat flour medium	X	X	-	-
Bengal gram medium	12. 32d	8. 35c	84. 2b	74. 6b
NA medium-II	38. 74b	25. 62b	97. 2a	94. 3a
Meat extract medium	X	X	-	-
Chicken offal medium	X	7. 12c	-	75. 4b
Modified wheat flour medium	10. 62c	6. 78c	80. 7b	69. 2c

The *S. siamkayai* were multilplied on 8 media out of 10 tried. 100% mortality were attained on Modified dog biscuit medium-I and NA-I medium. The infective juveniles produced from Wout's medium caused 99. 2% mortality followed by 97. 9% mortality in NA medium-II. In Bengal gram, Chicken offal medium and modified wheat flour medium, least mortality of 85. 5 and 80. 2 were attained. This study showed that *S. siamkayai* multiplied on different media and it also correlates with the results obtained by (Umamaheswari *et al*., 2008) [30]. In that, Maximum multiplication of *S. siamkayai* (501 x 103 IJs) was observed in Wout's medium followed by medium containing Bengal gram flour and groundnut oil.

The *H. indica* multiplied on 7 media out of 10 tried. 100% mortality were attained on 2 media *viz*., Egg yolk-I and NA-I media. These media produced significantly higher number of infective juveniles than other media. 95. 5%, 95. 3% and 92. 8 mortality was attained on Wout's media and NA media-II. Modified dog biscuit. Least production of 79. 5 and 74. 8 were reproduced on Bengal gram medium and Chicken offal medium. Hussaini *et al.* (2002) [20] reported that four media, *viz*., Wout's medium, modified egg yolk, soyflour + cholesterol and modified dog biscuit media yielded highest number of *S. carpocapsae*, *S. tami* 2. 1 and *H. indica*

Test media	Infective juveniles released per 250 ml flask		Mortality of insects	
	S. siamkayai	***H. indica***	***S. siamkayai***	***H. indica***
Wout's medium	28. 62b	26. 58b	97. 2a	95. 5a
Egg yolk-I	34. 68b	39. 24b	99. 2a	100a
NA medium- I	48. 09a	40. 20a	100a	100a
Modified Dog biscuit medium	52. 90a	26. 04b	100a	92. 8a
Wheat flour medium	X	X	-	-
Bengal gram medium	14. 65d	12.. 39d	85. 5b	79. 5b
NA medium-II	37. 55b	25.. 80b	97. 9a	95. 3a
Meat extract medium	X	X	-	-
Chicken offal medium	11. 23d	8. 33c	80. 2b	74. 8b
Modified wheat flour medium	13. 35d	X	83. 3b	-

X- No multiplication; Means followed by the same letter in columns are not significantly different at $P<0.05$.

Comparative virulence of *H. indica* and *S. glaseri* multiplied *in vivo* and *in vitro*

Comparative virulence of *in vitro* cultured nematodes multiplied in five different media *viz*., Wout's, dog biscuit and modified egg yolk medium, Bengal gram medium, NA medium-I *in vivo* cultured nematodes were tested on the final instar larvae of *C. cephalonica*.

Median Lethal Concentration (LC_{50}) Median Lethal Time (LT_{50})

In vivo multiplied infective juveniles were more virulent than *in vitro* multiplied infective juveniles. Even though, *in vitro* methods have been developed for mass production of some entomopathogenic nematodes, the virulence of the same has not been compared with that of infective juveniles produced on a host.

H. indica

The median lethal concentration (LC_{50}) of *in vivo* culture was minimum (8. 03 IJ/ larva). Among the five different media, lowest LC_{50} value was observed in NA media-I with a value of (13. 56 IJ/larva) followed by Modified dog biscuit media (15. 83), Modified egg yolk medium (18. 67), Wout's media (21. 42), Bengal gram medium (23. 52) The median lethal time (LT_{50}) also indicated that *in vivo* culture was more virulent (LT_{50} 34. 06 h/larva) than *in vitro* culture (LT_{50} 442. 04 h/ larva) respectively. House *et al*. (1964) used dog biscuits for preparing a medium for production of entomopathogenic nematodes. The method yielded 3 x 10^5 to 7. 1 x 10^6 IJs/plate. Among *in vivo* and *in vitro* multiplication, the best one was found to be *in vivo* followed by *in vitro* method (Table 5)

Table 5: Median lethal concentration (LC50) of *H. indica* multiplied *in vivo* and *in vitro* against final instar larvae of *C. cephalonica*

Medium	Chi Square Value	B Value	LC 50	Fiducial Limit	
				Lower	Upper
In vivo (C. cephalonica)	5. 88	1. 26	8. 03	6. 06	9. 89
In vitro (Modified dog biscuit medium)	7. 61	1. 34	13. 56	9. 22	12. 37
Modified egg yolk	7. 65	1. 42	18. 67	15. 32	23. 02
Wout"s medium	7. 70	1. 53	21. 42	19. 24	26. 44
Bengal gram medium	8. 32	1. 65	23. 52	20. 01	32. 94
NA medium-I	6. 05	1. 22	15. 83	7. 05	10. 63

Medium	Chi Square Value	B Value	Lt 50	Fiducial Limit	
				Lower	Upper
In vivo (C. cephalonica)	5. 88	3. 84	34. 06	22. 25	29. 41
In vitro (Modified dog biscuit medium)	7. 42	3. 86	36. 05	26. 56	32. 11
Modified egg yolk	7. 67	3. 89	38. 08	34. 12	40. 28
Wout"s medium	7. 92	4. 01	40. 07	36. 14	42. 12
Bengal gram medium	9. 32	4. 05	42. 04	38. 55	43. 11
NA medium-I	7. 02	3. 18	36. 12	23. 78	22. 14

S. glaseri

The median lethal concentration (LC_{50}) of *in vivo* culture was minimum (7. 55 IJ/ larva). Among the three different media, lowest LC_{50} value was observed in NA Media-I with a value of (11. 07IJ/larva) followed by Modified dog biscuit media (13. 44 IJ/larva), Modified egg yolk medium (19. 52 IJ/larva), Wout's media (21. 25), Bengal gram medium (23. 52). The median lethal time (LT_{50}) also indicated that *in vivo* culture was more virulent (LT_{50} 23. 42 h/larva) than *in vitro* culture (LT_{50} 42. 65 h/ larva) respectively. (Table 6) Among *in vivo* and *in vitro* multiplication, the best one was found to be *in vivo* followed by *in vitro* method.

Table 6: Median lethal concentration (LC50) of *S. glaseri* multiplied *in vivo* and *in vitro* against final instar larvae of *C. cephalonica*

Medium	Chi Square Value	B Value	LC 50	Fiducial Limit	
				Lower	Upper
In vivo (*C. cephalonica*)	4. 65	1. 01	7. 55	5. 64	7. 81
In vitro (Modified dog biscuit medium)	5. 95	1. 55	13. 44	7. 44	12. 69
Modified egg yolk	6. 64	1. 99	19. 52	13. 45	20. 11
Wout"s medium	6. 43	1. 67	21. 25	10. 13	16. 07
Bengal gram medium	8. 32	2. 03	23. 52	20. 01	32. 94
NA medium-I	4. 88	1. 32	11. 67	5. 76	8. 99

Medium	Chi Square Value	B Value	LT 50	Fiducial Limit	
				Lower	Upper
In vivo (*C. cephalonica*)	7. 01	5. 05	23. 42	21. 25	25. 81
In vitro (Modified dog biscuit medium)	7. 67	4. 89	29. 31	24. 59	30. 54
Modified egg yolk	8. 05	4. 27	42. 51	35. 75	36. 28
Wout"s medium	7. 81	4. 55	32. 92	27. 22	32. 11
Bengal gram medium	8. 94	5. 01	42. 65	38. 55	43. 11
NA medium-I	7. 55	5. 28	26. 78	22. 76	27. 89

Conclusion

Among the 4 strains of entomopathogenic nematodes, *S. glaseri* and *H. indica* found to be a highly virulent followed by *S. siamkayai*, *H. bacteriophora*. Highest yield of nematodes were achieved in *in vivo* production. In *in vitro* production, Egg yolk –I, NA medium-I and Modified dog biscuit medium produced more number of infective juveniles on *S. glaseri, H. bacteriophora, S. siamkayai, H. indica.*

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