

THE EVALUATION OF MULTIPLICATION CAPACITY IN *GALLERIA* OF ENTOMOPATHOGENIC NEMATODE ISOLATES FROM VIETNAM

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ABSTRACT: A total of 43 isolates of entomopathogenic nematodes of Vietnam were assessed on reproduction capacity in the last instar larvae of great wax moth (*Galleria mellonella*). The total yield of infective juveniles (IJs) on the *Galleria mellonella* were varied between indigenous isolates with significant different of multiplication. The amounts of IJs were produced in each *Galleria* from several thousands to hundreds thousands. These were shown a great difference of the reproduction capacity between indigenous isolates/species of entomopathogenic nematodes. In general, the multiplications of *Steinernema* isolates were lower than the multiplication of *Heterorhabditis* isolates. Among *Steinernema* isolates, however, the multiplications of some isolates were relatively low such as isolate S-DL9 (*S. eapokense*) with $4,378 \pm 509$ IJs, whereas other ones were relatively high such as S-TX1 (*S. sangi*) with $68,343 \pm 8,942$ IJs. These were also occurred significant difference between isolates within species that were particularly happened isolates of *Heterorhabditis indica* with average yields varieties from 18,900 IJs at H-PP16 to $233,500 \pm 39,100$ at H-NT3. The experiments showed the relationship between multiplication capacity of IJs and inoculation concentration and each isolate has an optimal inoculation concentration for highest multiplication. These data to be provided the important information for evaluation of potential isolates for biocontrol.

Keywords: Entomopathogenic nematodes, reproduction in *G. mellonella*, *Steinernema*, *Heterorhabditis*, Vietnam.

Entomopathogenic nematode species (EPN) of two genera Steinernematidae and Heterorhabditidae are obligated parasites in insects and they are usually infecting and killing a broad range of insect species. These characters of EPN have been used them as prospective biological control agents of insect pests in a variety of crops [3]. As naturally, each species/strains of EPN has usually linked with several their host insects whose relationships have been informed long time together with their local geographical-climate distributions. Therefore, the indigenous isolates/strains of nematodes have several merits to local insect pests.

The survey and sampling of indigenous EPN is not only provided information on EPN distribution but these is also supplied potential isolates/strains which might be more suitable for the inundation release against local insect

pests because of adaptation to local climate and population regulators. Even the information on available of indigenous EPN is very useful for any decision prior to possible introduction of exotic species. Since benefit of the indigenous EPN, the survey and collection works on EPN have not only limited in the Europe, North America and Australia but have also been extended to Asian, African and Latin American countries. These leading to increasing number of EPN isolates/species [2]. The number of EPN surveys in tropical and subtropical regions has significantly increased during last decade.

The survey and isolation of entomopathogenic nematodes in Vietnam were started in 1997. Up to now, there are about fifty of entomopathogenic nematode isolates isolated and maintained by living cultures in the Laboratory. Most of entomopathogenic nematodes previously were unknown and their

comprehensive studies based on morphological and molecular characterization as well as conducting some cross breeding tests allowed imaging a picture of the biodiversity of EPN in Vietnam [7, 9-12]. Concurrently with taxonomy works, the pathogenicity tests based on the indigenous isolates were obtained with *Galleria mellonella* and then with some insect pests to evaluate the potential of indigenous isolates for biological control. Recently, almost results of the pathogenicity tests on insect pests were published [4-6]. In the scope of experimental tests in the laboratory condition, the reproduction capacity of indigenous EPN isolates were assessed and evaluated. The pathogenicity and reproduction capacity are usually allowed the using potential isolates for biological control.

The present paper is given the assessment on reproduction capacity of indigenous isolates of entomopathogenic nematodes in Vietnam. Most of the studies were conducted in Vietnam during years 1999-2003 and some further examined experiments were carried out in the Laboratory of Biotechnology and Biocontrol, Kiel University, Germany in 2005 before their deposit in liquid nitrogen.

II. MATERIAL AND METHOD

A collection of living entomopathogenic nematodes which consists of 43 isolates belonging to 18 entomopathogenic species has been maintained in the Department of Nematology, Institute of Ecology and Biological Resources. All these isolates were used for the assessment of the multiplication potential on last instars larvae of great wax moth, *G. mellonella*. Apart from indigenous isolates some introduced isolates such as DL36 (*Steinernema carpocapsae*), *S. arenaria*, HP88 (*Heterorhabditis bacteriophora*) has been used for the competition.

For maintaining cultures of entomopathogenic nematodes: each isolate was periodically cultured depending their survival time in the storage temperature at 14°C that condition allowed survival of *Steinernema* isolates for approximately 180 days and that were app. 90 days for survival of *Heterorhabditis* isolates. Inoculation concentration was 100 IJs/*Galleria*.

Testing procedure for estimation of best-fit concentration of EPN inoculation for highest EPN multiplication capacity: each EPN isolates was conducted with ten different concentrations of IJs that were between 10 and 100 IJs/*Galleria*. Each concentration formula was used ten last instars larvae of *G. mellonella* for inoculation in a 9cm diameter petri dish which is transferred 1 ml of distilled water contained following concentration 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 IJs on a Whatman filter paper then placed 10 *Galleria* for exploring for 48 hours in room temperature. All dead *Galleria* by EPN was determined and cadavers were transferred to another wetted Whatman paper putted into Petri dish for incubation during 4-10 days. Collection of IJs by water trap [14] and IJs were calculated with a counting dish under stereomicroscope. The multiplication experiment was repeated at least five times in the same condition. Average of IJs produced on each *Galleria* was statistically processed following Anon with correlation index (R^2) to estimate goodness-of-fit-test of optimal concentration for each EPN with highest multiplication.

III. RESULT AND DISCUSSION

1. The reproduction capacity in *Galleria* of indigenous entomopathogenic nematode isolates

The reproduction character and its multiplication capacity of entomopathogenic nematode isolates can be considered as important category of a potential agent for biological control. The high multiplication capacity of any EPN isolate to be not only expressed a high sensitive of the insect host to EPN isolate, but it's allowed the culture of EPN with high production.

In our experiments when EPN isolates were periodically cultured for maintenance purpose, the total IJs yield of indigenous isolates produced per *Galleria* were significantly variable in a range between several thousands and hundreds thousands among different species of these indigenous entomopathogenic nematodes. In general, the total yields of *Steinernema* isolates and species were less than those of *Heterorhabditis* isolates. Among *Steinernema* isolates the yield of some isolates were relatively low such as S-DL9 (*Steinernema* sp.1), S-TS2 (*S.*

sasonense), S-DL14 (*S. cumgarensis*), S-DL23 (*Steinernema* sp.1) with 4,378 ± 509 IJs, 5,000 ± 600 (3,800-5,700) IJs, 5,500 ± 300 (5,100-5,900) IJs, 5,700 ± 500 (5,100-6,600) IJs, respectively. These yields however were relatively high at some isolates such as S-TX1, S-XS4 (*Steinernema sangi*), S-TK10 (*Steinernema loci*), S-TN10 (*S. robustispiculum*) and S-XT (*Steinernema* sp.6) with 68,343 ± 8,942 (55,900-75,100) IJs, 36,600 ± 2,200 (34,300-39,600) IJs, 34,400 ± 5,200 (31,800-40,400) IJs, 32,000 ± 1,100 (30,500-33,100) IJs and 30,100 ± 900 (28,800-31,500) IJs, respectively.

The reproduction capacities were not only occurred with larger varieties between species and isolates among the genera and inside each genus but also occurred even very much

different between isolates within the species. The difference was particularly found within isolates of *Heterorhabditis indica* species with average yields from 18,900 ± 400 (18,400-19,800) IJs at H-PP16 to 233,500 ± 39,100 (218,600-269,900) at H-NT3. In the *Steinernema sangi* species this broad variation was occurred between three isolates with the average yields from 7,000 ± 500 (6,300-7,700) IJs to 68,343 ± 8,942 (55,900-75,100) IJs. In case of species *S. robustispiculum* with four experimented isolates the reproduction capacity was significantly varied from 18,600 ± 1,600 to 22,500 IJs. Some other examples on the difference of reproduction capacity were occurred with the species of *S. sasonense* when their average multiplication yields were varied between 5,000 to 19,800 IJs per *Galleria* (table 1).

Table 1

Infective juvenile yields of 43 indigenous entomopathogenic nematode isolates of Vietnam
(in the laboratory conditions)

N	Species	Strains	IJs yield per <i>Galleria</i> (× 1000)
1.	<i>H. indica</i>	H-BB9	24.0 ± 0.5 (23.4-25.1)
2.	<i>H. indica</i>	H-CP16	127.2 ± 7.0 (124.4-128.1)
3.	<i>H. indica</i>	H-CP6	26.5 ± 0.4 (25.8-27.3)
4.	<i>H. indica</i>	H-CP8	27.3 ± 0.8 (26.4-28.5)
5.	<i>H. indica</i>	H-HS5	24.4 ± 0.6 (23.3-25.2)
6.	<i>H. indica</i>	H-MP11	180.8 ± 3.2 (178.0-184.4)
7.	<i>H. indica</i>	H-NT3	233.5 ± 39.1 (218.6-269.9)
8.	<i>H. indica</i>	H-PP16	18.9 ± 0.4 (18.4-19.8)
9.	<i>H. indica</i>	H-QB2	31.9 ± 0.8 (30.9-33.4)
10.	<i>H. indica</i>	H-QB3	32.1 ± 0.6 (31.5-33.6)
11.	<i>H. indica</i>	H-TN48	24.3 ± 0.6 (23.5-25.1)
12.	<i>S. eapokense</i>	S-DL16	7.8 ± 0.9 (6.4-8.8)
13.	<i>S. eapokense</i>	S-DL20	5.8 ± 0.4 (5.4-6.7)
14.	<i>S. eapokense</i>	S-DL21	6.1 ± 0.5 (5.3-6.8)
15.	<i>S. eapokense</i>	S-DL22	6.1 ± 0.4 (5.3-6.5)
16.	<i>S. cumgarensis</i>	S-DL13	6.4 ± 0.4 (5.6-6.9)
17.	<i>S. cumgarensis</i>	S-DL14	5.5 ± 0.3 (5.1-5.9)
18.	<i>S. sangi</i>	S-MP9	7.0 ± 0.5 (6.3-7.7)
19.	<i>S. sangi</i>	S-TX1	68.3 ± 8.9 (55.9-75.1)
20.	<i>S. sangi</i>	S-XS4	36.6 ± 2.2 (34.3-39.6)
21.	<i>S. robustispiculum</i>	S-TN10	32.0 ± 1.1 (30.5-33.1)
22.	<i>S. robustispiculum</i>	S-TN24	18.6 ± 1.6 (16.4-22.1)
23.	<i>S. robustispiculum</i>	S-TN21	23.2 ± 1.9 (20.3-25.7)
24.	<i>S. robustispiculum</i>	S-TN22	23.4 ± 1.3 (21.2-25.1)
25.	<i>S. sasonense</i>	S-TN53	19.8 ± 0.7 (18.7-21.2)
26.	<i>S. sasonense</i>	S-TS2	5.0 ± 0.6 (3.8-5.7)

27.	<i>S. sasonense</i>	S-TS10	19.4 ± 0.6 (18.5-20.2)
28.	<i>Steinernema loci</i>	S-TK10	34.4 ± 5.2 (31.8-40.4)
29.	<i>S. thanhi</i>	S-TG10	24.7 ± 0.4 (23.9-25.2)
30.	<i>Steinernema</i> sp.B	S-TD16	7.5 ± 0.4 (6.9-8.3)
31.	<i>Steinernema</i> sp.B	S-TD6	8.9 ± 0.7 (7.5-10.2)
32.	<i>Steinernema</i> sp.C	S-BM12	22.5 ± 0.6 (21.3-23.3)
33.	<i>Steinernema</i> sp.E	S-TN9	31.5 ± 1.1 (30.2-33.0)
34.	<i>Steinernema</i> sp.E	S-TN38	22.3 ± 0.7 (21.2-23.6)
35.	<i>Steinernema</i> sp.J	S-CP13	28.4 ± 1.5 (25.4-30.2)
36.	<i>Steinernema</i> sp.J	S-CP14	27.3 ± 1.7 (24.3-30.1)
37.	<i>Steinernema</i> sp.1	S-DL23	5.7 ± 0.5 (5.1-6.6)
38.	<i>Steinernema</i> sp.1	S-DL9	4.4 ± 0.5 (3.6-5.3)
39.	<i>Steinernema</i> sp.2	S-NH7	6.4 ± 0.3 (6.1-6.8)
40.	<i>Steinernema</i> sp.3	S-BC	6.2 ± 0.5 (5.3-6.7)
41.	<i>Steinernema</i> sp.4	S-CP12	6.9 ± 0.7 (5.5-7.7)
42.	<i>Steinernema</i> sp.5	S-HS2	9.2 ± 0.6 (8.3-10.2)
43.	<i>Steinernema</i> sp.6	S-XT	30.1 ± 0.9 (28.8-31.5)

The difference on reproduction capacity between entomopathogenic nematode isolates among species of the genus and within the isolates of each species might be depended on natural habitat and almost related with insect host in the nature. In the Laboratory condition, the multiplication experiment tests were carried out with the same insect host as great wax moth, *Galleria mellonella* so most of them might be not affected by natural insect hosts. Because the multiplication of each EPN isolates is as result of the complex interactions between parasitic nematodes and their symbiotic bacterium on each insect host, these interactions of nematode-bacterium lead to different reproduction and it can be interpreted of how different multiplication is between EPN isolates/species.

In comparison with some other introduced strains that were evaluated and screened as agents for biological control such as D-36 (*S. carpocapsae*), *S. arenaria* and HP88 (*H. bacteriophora*), in the same of culturing conditions, some indigenous isolates were shown the multiplication with an equivalent level or even somewhat higher yield such as H-NT3, H-MP11 and H-CP16 (*H. indica*) with average yield from 233,500 IJs, 180,800 IJs and 127,200 IJs, respectively. In genus *Steinernema*, isolate S-XT (*Steinernema* sp.6), S-TN9 (*Steinernema* sp.E), S-TK10 (*S. loci*),

S-XS4, S-TX1 (*S. sangi*) were found with average yield from 30,140 IJs, 31,960 IJs, 34,370 IJs, 36,340 IJs and 68,340 IJs, respectively. The reproduction yields of *Steinernema* isolates of Vietnam were less than data on multiplication capacity of *S. feltiae* conducted by Dutky et al. [1] on *Galleria* with 200,000 IJs/*Galleria*. However, it was clearly that in most of *Steinernema* isolates/species which had been valued for biological control, the multiplication capacity varied between 30,000-50,000 IJs [13]. In other experiment on valuation of reproduction capacity of *H. bacteriophora*, the multiplication was yielded with 35,000 IJs [8]. Thus the multiplication of entomopathogenic nematodes inside insect host as *Galleria* might be depended on biological property of EPN strain and their intinial infective density. Apart from that, normally for any EPN strain with smaller size of body as almost strain of *Heterorhabditis* spp., their multiplication yields are trended as higher than multiplication yields of strains with larger size of body such as almost strains of *Steinernema* species.

Although these tests are conducted in the laboratory condition on standard insect host with *Galleria* as frequency, their data on reproduction are being valuable assessments on toxicological level concerning mass producing of specific strain of symbiosis bacterium. Consequently, based on these data of reproduction yields, some

indigenous with moderate high multiplication has been evaluated as potential strains and their further assessments on their optimal reproduction and their pathogenicity on insect pests are need to be conducted.

2. Relation between final yield and concentration of infested IJs

In each EPN isolate, the highest reproduction yield was depending on inoculation concentration of infective juvenile of each tested isolates. In our assays carried

out with four indigenous isolates, the highest yields of all isolates were obtained with inoculated concentration of IJs from 60-70 IJs of S-TK10 and S-TX1 (*Steinernema* spp.) per *Galleria* (table 2). The relation between yield and infested IJs in all assays showed clearly with a close correlation (fig. 1 and 2). This mean that each isolate is being with the highest yield or highest multiplication that corresponded with a certainly inoculated concentration of IJs that is usually considered as the best fit inoculated concentration for each EPN isolate.

Table 2

The relationship between yield and inoculated concentration of trains S-TK10 and S-TX1 (temp: 26.1-29.4°C; humidity: 77-86%)

Infested IJs	Number IJ per <i>Galleria</i> (× 1000)	
	<i>Steinernema loci</i> (TK10)	<i>Steinernema sangi</i> (TX1)
10	12.1 ± 4.0 (8.1-16.1)	35.7 ± 13.9 (27.1-51.7)
20	18.5 ± 3.5 (15.0-22.0)	37.1 ± 13.5 (21.4-44.5)
30	19.6 ± 2.8 (16.8-22.5)	33.4 ± 6.4 (29.5-40.8)
40	23.8 ± 13.8 (14.8-39.7)	50.1 ± 4.0 (45.5-52.9)
50	26.9 ± 1.6 (25.9-28.8)	55.7 ± 7.5 (49.3-64.0)
60	27.8 ± 7.9 (22.7-37.0)	55.8 ± 12.9 (41.5-66.6)
70	34.4 ± 5.2 (31.8-40.4)	54.5 ± 7.6 (45.9-60.3)
80	23.9 ± 4.4 (19.8-28.5)	49.9 ± 6.0 (44.4-56.3)
90	29.6 ± 5.6 (23.6-34.9)	47.1 ± 9.8 (37.6-57.2)
100	22.7 ± 6.5 (15.7-28.4)	53.8 ± 10.0 (45.3-64.8)

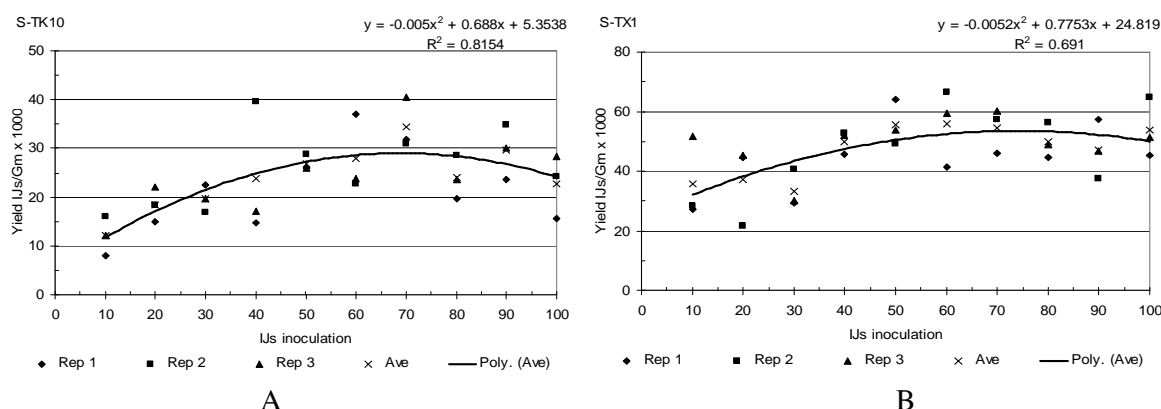


Figure 1. Correlation between IJs yields and concentration of infested IJs
A. S-TK10 (*Steinernema loci*); B. S-TX1 (*Steinernema sangi*).

This similarity is also occurred with two strains of the species *Heterorhabditis indica* when the optimal of the inoculated concentration of IJs was 50 IJs at H-MP11 and 60 IJs at H-NT3 per *Galleria* (table 3). The yield curves may be in both cases were moderate

regulation and the correlation value was also tight in H-MP11 but in case of H-NT3 it dropped points of production yields which were not too much close to the curve.

It is evidently that in the same culture condition but there was some difference

appeared about multiplication yield of the same isolate. This might be interpreted on the

dependant of inoculation concentration of each isolate.

Table 3

The relationship between yield and inoculated concentration of trains H-MP11 and H-NT3
(temp: 26.1-29.4°C; humidity: 77-86%)

Infested IJs	Number IJ per <i>Galleria</i> (× 1000)	
	<i>Heterorhabditis indica</i> (MP11)	<i>Heterorhabditis indica</i> (NT3)
10	140.3 ± 6.2 (134.2-146.6)	137.4 ± 33.1 (101.7-167.0)
20	150.4 ± 10.8 (142.6-162.7)	139.4 ± 15.9 (122.0-153.2)
30	162.5 ± 7.8 (153.9-169.0)	151.5 ± 20.4 (130.5-171.2)
40	166.4 ± 13.1 (151.4-174.3)	176.1 ± 15.9 (162.0-193.3)
50	180.8 ± 3.2 (178.0-184.4)	229.7 ± 28.1 (204.0-259.7)
60	171.6 ± 6.3 (164.4-176.3)	233.5 ± 39.1 (238.6-269.9)
70	162.6 ± 7.4 (154.4-168.9)	167.1 ± 19.1 (149.6-187.6)
80	161.9 ± 6.9 (156.6-169.7)	141.3 ± 14.3 (129.7-157.3)
90	152.4 ± 10.5 (141.2-162.1)	139.1 ± 13.7 (128.0-154.4)
100	147.4 ± 8.7 (141.2-157.3)	130.4 ± 15.5 (112.9-142.4)

The difference on value of the standard deviation between isolates was indicated the stable level of multiplication yield for each EPN isolate. The high value of standard deviation implies that the multiplication yield of any isolate is less stable than those of low value of standard deviation. This also mean that most of isolates with low multiplication yield and high value of standard

deviation were less stable whereas isolates with high multiplication yield and low value of standard deviation were usually more stable. In addition, some isolates with low multiplication yield is usually difficult for culturing and maintaining. This interpreted that the EPN isolate might be not too much compatible with the insect host as *Galleria* other than its natural host.

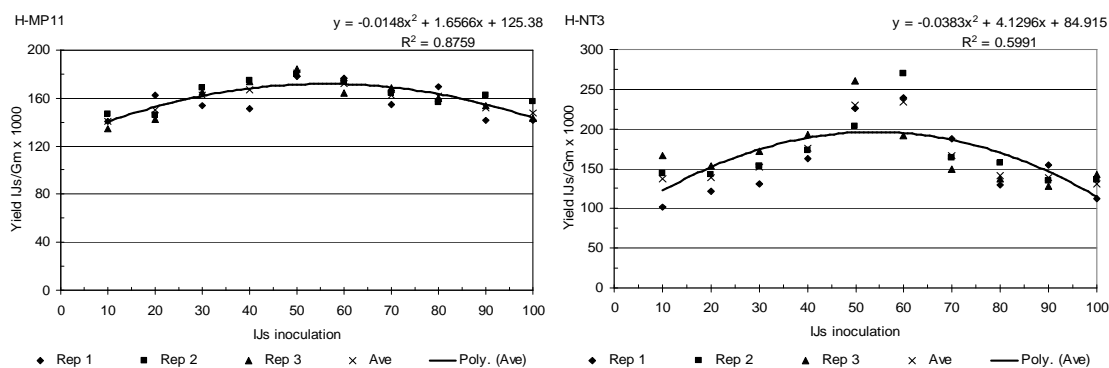


Figure 2. Correlation between IJs yields and concentration of infested IJs
A. H-MP11; B. H-NT3 (*Heterorhabditis indica*).

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ĐÁNH GIÁ KHẢ NĂNG SINH SẢN TRÊN *GALLERIA* CỦA CÁC CHỦNG TUYẾN TRÙNG KÝ SINH GÂY BỆNH CÔN TRÙNG Ở VIỆT NAM

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TÓM TẮT

Tổng số 43 chủng tuyến trùng ký sinh gây bệnh côn trùng bản địa Việt Nam đã được đánh giá khả năng sinh sản trong ấu trùng bướm sấp lớn (*Galleria mellonella*). Sản lượng thu hoạch của ấu trùng cảm nhiễm (IJs) trên *Galleria* thay đổi giữa các chủng tuyến trùng với sự khác biệt về sinh sản lớn. Số lượng ấu trùng cảm nhiễm được sinh ra trong mỗi *Galleria* từ một vài ngàn đến hàng trăm ngàn. Điều này chứng tỏ có sự khác nhau lớn về khả năng sinh sản giữa các chủng/các loài tuyến trùng ký sinh gây bệnh côn trùng. Nhìn chung, sự sinh sản của các chủng *Steinernema* thấp hơn so với các chủng *Heterorhabditis*. Trong số các chủng *Steinernema* thì một số chủng có sản lượng IJs tương đối thấp như S-DL9 (thuộc loài *S. eapokense*) với sản lượng IJs trung bình chỉ đạt 4.378 ± 509 IJs, trong khi đó một số chủng có sản lượng IJs tương đối cao như S-TX1 (loài *S. sangi*) với sản lượng IJs trung bình đạt 68.343 ± 8.942 IJs. Sự khác nhau này cũng xảy ra đối với các chủng EPN trong cùng loài, đặc biệt ở các chủng của loài *Heterorhabditis indica* với sản lượng IJs trung bình thay đổi từ 18.900 IJs ở chủng H-PP16 đến 233.500 ± 39.100 ở chủng H-NT3. Các thí nghiệm cho thấy có mối tương quan chặt chẽ giữa nồng độ gây nhiễm và khả năng sinh sản của các chủng tuyến trùng, trong đó mỗi chủng tuyến trùng có một sản lượng IJs cao nhất ứng với một nồng độ gây nhiễm tối ưu. Số liệu về khả năng sinh sản của các chủng EPN cung cấp thông tin quan trọng cho việc đánh giá và tuyển chọn các chủng tuyến trùng tiềm năng cho phòng trừ sinh học sâu hại.

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