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## Biology and Life Table Studies on Healthy and *Pasteuria penetrans* Infected *Meloidogyne incognita*

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### ABSTRACT

#### Keywords

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*Pasteuria penetrans* is an important parasite of *Meloidogyne incognita*. Lifecycle of this parasite is in close association with the nematode. Life table was constructed for healthy and *P. penetrans* infected population of *M. incognita*. Results revealed that the life cycle of *P. penetrans* infected *M. incognita* was extend by 3 days when compared to healthy population. Survival and life expectancy of the infected population was reduced by 2 days. Stable age distribution revealed that reduction in survival of infected population in all stages. Fecundity rate was reduced to four folds compared to healthy population. This subsequently reduced the population doubling time.

### Introduction

A global agricultural lose of \$157 billion was annually incurred due to plant parasitic nematodes (Abad *et al.*, 2008). Root knot nematode *Meloidogyne incognita* is an economically important parasite. Life table is a systematic study of survival and mortality of a population. It is used to determine whether a population is growing, declaning or remaining

stale. It is highly useful in studying factors responsible for change in population size (Atwal and bains 1974).

It is used to determine whether a population is growing declining or remaining stable. It gives a knowledge on rate of development of an organism, age specific fecundity. This stimulates the outcome of management decessions. Many authors had developed life

table studies for many nematodes viz. *M. arenaria* (Ferris and Hunt 1979) *Heterodeara cajani* (Singh and Sharma 1995). The present study attempted to compare the life table of healthy and *Pasteruia penetrans* infected *M. incognita* population on tomato.

**Materials and Methods**

Seeds of tomato cv.Co3 were sown in a seedling pan. After germination the seedlings of equal height were transplanted to 80 tumbler pots (40 for infected and 40 for healthy nematodes) of 7 cm diameter containing steam sterilized soil. The seedlings were allowed to establish for 10 days. *P. penetrans* encumbered J2 were prepared by adding fresh spores extracted from white females. Healthy J2 were centrifuged along with spores of *P. penetrans* in 1.5ml eppendorf tubes at 500rpm for 2 min. The nematodes were examined for 100 per cent encumbrance under steriozoom microscope. Freshly hatched healthy and *P. penetrans* infected juveniles at cohort were inoculated at

the rate of 400 J2 per pot. Nematode development and age specific survival were studied by removing the seedlings at every 24 hours after inoculation. The roots were stained with acid fuchsin-lactophenol and destined in plain lactophenol. The roots were compressed between two glass plates (15x10 cm) and the number of juveniles in different stage was counted under a stereo zoom microscope. If the internal body content of the nematode is not intact, they were considered as dead nematodes. Peak occurrence of each life stage was used to calculate survival and development of different stages. The procedure was continued till adult females were developed. All data were corrected and adjusted in such a way that the life table commences with a cohort of 100 J2 as described by Singh and Sharma (1994). Life table was constructed as described by Deevey (1947). The fecundity and intrinsic rate of natural increase of population were calculated by using Birch (1948) formula as elaborated by Howe (1953). The following columns were used.

X	Pivotal age days
$l_x$	Age specific longevity
$m_x$	Age specific fecundity
$R_0(l_x, m_x)$	Net reproductive rate
$rm = \ln R_0 / T_c$	Innate capacity for increase of females per female per day)
X ( $d_x$ )	Number of dying individual in cohort
$1000q_x = d_x / l_x * 100$	Per cent apparent mortality
$100dx/n$	Per cent real mortality
$L_x = l_x + (x+1)/2$	Number alive between age x and x+1
$X T_x$	Number of individual life days beyond age
$e_x = T_x / l_x$	Expectation of further life days
$\sum m_x$	Gross Reproductive Rate (GRR)
$R_0 = \sum l_x m_x$	Net Reproductive Rate (NRR)
$T_c = (\sum l_x m_x) / R_0$	Mean Length of Generation (MLG)
$R_c = (\log R_0) / T_c$	Innate capacity for increase in number)
$T = (\ln R_0) / rm$	Corrected generation time in days
$R_m = e^{7-rm_x} \cdot l_x m_x$	Intrinsic rate of increase
$T = (\log_e R_0) / rm$	Corrected generation time days
$\Lambda = e^{rm}$	Finite rate of increase
$C = (e^{-rmx}) / \sum e^{-rmxlx}$	Stable age distribution

## Results and Discussion

### Biology of *M. incognita*

The biology of spore encumbered J2 was extended by 3 days compared to healthy J2. The results revealed that survival of J2 was increased by *P. penetrans* infection. The spore burden on the J2 make them difficult to penetrate the host and only J2 which have few spore reached the host. J2 with heavy spores attachment survived till the food reserves in them are depleted (Stirling, 1984).

Competition and other factors might weaken the J2 and predispose them to infection by microorganisms (Davies *et.al.*, 1988; Davies, 2009). Infected J2 which successfully reached the host became J3. The duration of infected J3 was extended by one day compared to healthy J3. Subsequently this led to increase in the duration of J4 by one day. Oviposition period was also increased by 2 days. Increase

in life cycle of *P. penetrans* infected *M. incognita* lead to increase in total life cycle by 3 days. Further the bacterium reduced the fecundity of *M. incognita* by 5 fold compared to healthy female populations. However, *P. penetrans* was not found to cause any morphological changes in the host. (Davies *et al.*, 2011) (Table 1).

### Survival and life expectancy of *M. incognita* infected by *P. penetrans*

The study on life table provides a concise summary of certain vital population statistics (Brich, 1948). According to Southwood (1976) it is the most useful numerical aid in studying population biology enabling determination of age distribution and mortality in natural populations of any organisms. Life table gives a vivid picture on the fecundity and growth potential of any organism under prevailing environmental conditions.

**Table.1 Biology of healthy and *P. penetrans* infected *M. incognita***

Parameters	Duration of stages in healthy nematodes		Duration of stages in infected nematodes	
	Range (Days)	$\bar{X} \pm SE$	Range (Days)	$\bar{X} \pm SE$
<b>Incubation period</b>	3-5	4.5±0.57	3-5	<b>4.6±0.50</b>
<b>J<sub>2</sub></b>	4-5	4.7±0.1	8-10	<b>9.2±0.20</b>
<b>J<sub>3</sub></b>	4-5	4.4±0.1	5-6	<b>5.5±0.33</b>
<b>J<sub>4</sub></b>	3-4	3.7±0.1	4-5	<b>4.3±0.21</b>
<b>Preovipositional period</b>	1-3	2.4±0.57	1-3	<b>2.7±0.60</b>
<b>Oviposition period</b>	10-14	12.3±1.15	12-15	<b>14.3±0.88</b>
<b>Total development period (egg – adult)</b>	25-36	30±1.40	34-44	<b>39±1.34</b>
<b>Fecundity</b>	270-300	286±8.81	78-83	<b>80.3±3.26</b>
<b>Average temperature 28±5°C</b>				
<b>Average RH 70±5 %</b>				
<b>± represents SE of mean</b>				

**Table.2** Survival and life expectancy of *M. incognita* infected with *P. penetrans*

Age in day (X)	Numbers surviving in X ( $l_x$ )	Numbers dying in X ( $d_x$ )	Per cent apparent mortality $100q_x=d_x/l_x * 100$	Per cent real mortality $100d_x/n$	No. alive between age x and X +1 $L_x= l_x+( X +1)/2$	No. of individual life days beyond age X ( $T_x$ )	Expectation of further life days $e_x = T_x/l_x$
0	100	0	0.00	0.00	99	2326	23.26
1	98	2	2.04	2.00	95.5	2227	22.72
2	93	5	5.38	5.00	93	2131.5	22.92
3	93	0	0.00	0.00	92.5	2038.5	21.92
<b>Egg</b>	<b>93</b>	<b>7</b>	<b>7.62</b>	<b>7.00</b>	-	-	-
4	92	1	1.09	1.00	90.5	1946	21.15
6	85	4	4.71	4.00	84	1768.5	20.81
8	78	5	6.41	5.00	78	1604	20.56
10	74	4	5.41	4.00	74	1450	19.59
<b>J<sub>2</sub></b>	<b>74</b>	<b>19</b>	<b>23.40</b>	<b>19.00</b>	-	-	-
12	72	2	2.78	2.00	70	1303	18.10
14	68	0	0.00	0.00	68	1165	17.13
16	68	0	0.00	0.00	68	1029	15.13
<b>J<sub>3</sub></b>	<b>68</b>	<b>6</b>	<b>8.66</b>	<b>9.00</b>	-	-	-
17	68	0	0.00	0.00	68	961	14.13
18	68	0	0.00	0.00	68	893	13.13
19	68	0	0.00	0.00	68	825	12.13
20	68	0	0.00	0.00	68	757	11.13
<b>J<sub>4</sub></b>	<b>68</b>	<b>0</b>	<b>0</b>	<b>0.00</b>	-	-	-
21	68	0	0.00	0.00	68	689	10.13
22	68	0	0.00	0.00	68	621	9.13
23	68	0	0.00	0.00	68	553	8.13
24	68	0	0.00	0.00	68	485	7.13
25	68	0	0.00	0.00	68	417	6.13
26	68	0	0.00	0.00	64	349	5.13
27	60	8	13.33	8.00	58	285	4.75
28	56	4	7.14	4.00	52.5	227	4.05
29	49	7	14.29	7.00	45.5	174.5	3.56
30	42	7	16.67	7.00	36	129	3.07
31	30	12	40.00	12.00	28.5	93	3.10
32	27	3	11.11	3.00	24	64.5	2.39
33	21	6	28.57	6.00	18	40.5	1.93
34	15	6	40.00	6.00	15	22.5	1.50
35	15	0	0.00	0.00	7.5	7.5	0.50
Adult	0	15	0.00	15.00	0	0	0.00

**Table.3** Survival and life expectancy of healthy *M. incognita* on tomato

Age in day (X)	Number s surviving in X ( $l_x$ )	Number s dying in X ( $d_x$ )	Per cent apparent mortality $100q_x=d_x/l_x *100$	Per cent real mortality $100d_x/n$	No. alive between age x and X+1 $L_x= l_x+(X+1)/2$	No. of individual life days beyond age X $T_x$	Expectation of further life days $e_x = T_x/l_x$
0	100	0	0.00	0.00	99.5	2516	25.16
1	99	1	1.01	1.00	95.5	2416.5	24.41
2	92	7	7.61	7.00	92	2321	24.23
3	92	0	0.00	0.00	92	2229	24.23
<b>Egg</b>	<b>92</b>	<b>8</b>	<b>8.62</b>	<b>8.00</b>	-	-	-
4	92	0	0.00	0.00	91	2137	23.23
5	90	2	2.22	2.00	89	2046	22.73
6	88	2	2.27	2.00	88	1957	22.24
7	88	0	0.00	0.00	86.5	1869	21.24
8	85	3	3.53	3.00	85	1782.5	20.97
9	85	0	0.00	0.00	85	1697.5	19.97
<b>J<sub>2</sub></b>	<b>85</b>	<b>7</b>	<b>8.02</b>	<b>7.00</b>	-	-	-
10	85	0	0.00	0.00	84.5	1612.5	18.97
11	84	1	1.19	1.00	84	1528	18.19
12	84	0	0.00	0.00	84	1444	17.19
13	84	0	0.00	0.00	83	1360	16.19
<b>J<sub>3</sub></b>	<b>84</b>	<b>1</b>	<b>1.19</b>	<b>1.00</b>	-	-	-
14	82	0	0.00	0.00	82	1277	15.57
15	82	0	0.00	0.00	82	1195	14.57
16	82	0	0.00	0.00	82	1113	13.57
17	82	0	0.00	0.00	82	1031	12.57
<b>J<sub>4</sub></b>	<b>82</b>	<b>0</b>	<b>0</b>	<b>0.00</b>	-	-	-
18	82	0	0.00	0.00	82	949	11.57
19	82	0	0.00	0.00	82	867	10.57
20	82	0	0.00	0.00	81	785	9.57
21	80	2	2.50	2.00	80	704	8.80
22	80	0	0.00	0.00	80	624	7.80
23	80	0	0.00	0.00	80	544	6.80
24	80	0	0.00	0.00	80	464	5.80
25	80	0	0.00	0.00	80	384	4.80
26	80	0	0.00	0.00	75	304	3.80
27	70	10	14.29	10.00	65	229	3.27
28	60	10	16.67	10.00	57	164	2.73
29	54	6	11.11	6.00	48	107	1.98
30	42	12	28.57	12.00	40	59	1.40
31	38	4	10.53	4.00	19	19	0.50
Adult	0	38	0.00	38.00	0	0	

**Table.4** Stable age distribution of *Meloidogyne incognita* juveniles infected by *P. penetrans*

Age	$l_x$	$m_x$	$l_x m_x$	$X.l_x.m_x$	$e^{-r m x} . l_x$	Stable age distribution	Percentage distribution
1	1				0.8353	0.186281	
2	0.98				0.6837	0.152484	
3	0.93				0.5420	0.120867	<b>46</b>
4	0.93				0.4527	0.100957	
5	0.92				0.3740	0.083419	
6	0.89				0.3022	0.067406	
7	0.85				0.2411	0.053771	
8	0.83				0.1967	0.043857	
9	0.78				0.1544	0.034426	
10	0.78				0.1289	0.028755	<b>41</b>
11	0.74				0.1022	0.022786	
12	0.74				0.0853	0.019033	
13	0.72				0.0694	0.015468	
14	0.68				0.0547	0.012202	
15	0.68				0.0457	0.010192	<b>8</b>
16	0.68				0.0382	0.008513	
17	0.68				0.0319	0.007111	
18	0.68				0.0266	0.005939	
19	0.68				0.0222	0.004961	
20	0.68				0.0186	0.004144	<b>3</b>
21	0.68	5.2	3.54	74.26	0.0155	0.003461	
22	0.68	6.6	4.49	98.74	0.0130	0.002891	
23	0.68	6.8	4.62	106.35	0.0108	0.002415	
24	0.68	7.2	4.90	117.50	0.0090	0.002017	
25	0.68	10.1	6.87	171.70	0.0076	0.001685	
26	0.68	10.2	6.94	180.34	0.0063	0.001407	
27	0.6	6.3	3.78	102.06	0.0047	0.001037	
28	0.56	5.8	3.25	90.94	0.0036	0.000809	
29	0.49	5.5	2.70	78.16	0.0026	0.000591	
30	0.42	4.5	1.89	56.70	0.0019	0.000423	
31	0.3	3.7	1.11	34.41	0.0011	0.000252	
32	0.27	3.2	0.86	27.65	0.0009	0.000190	
33	0.21	2.6	0.55	18.02	0.0006	0.000123	
34	0.15	1.4	0.21	7.14	0.0003	0.000074	
35	0.15	1.2	0.18	6.30	0.0003	0.000061	
36	0	0	0	0	0.0000	0.000000	<b>2</b>

**Table.5** Stable age distribution of healthy *Meloidogyne incognita* juveniles

Age	$l_x$	$m_x$	$l_x m_x$	$X.l_x m_x$	$e^{-r m x} . l x$	Stable age distribution	Percentage distribution
1	1				0.8353	0.1804491	45
2	0.99				0.6907	0.1384722	
3	0.92				0.5361	0.1074836	
4	0.92				0.4478	0.0897779	79
5	0.92				0.3740	0.0749888	
6	0.9				0.3056	0.0612743	
7	0.88				0.2496	0.0500432	
8	0.88				0.2085	0.0417996	
9	0.85				0.1682	0.0337237	11
10	0.85				0.1405	0.0281684	
11	0.85				0.1174	0.0235282	
12	0.84				0.0969	0.0194212	
13	0.84				0.0809	0.0162220	6
14	0.84				0.0676	0.0135497	
15	0.82				0.0551	0.0110482	
16	0.82				0.0460	0.0092282	4
17	0.82				0.0384	0.0077081	
18	0.82	22.3	18.286	329.148	0.0321	0.0064383	
19	0.82	23	18.860	358.340	0.0268	0.0053777	
20	0.82	26.5	21.730	434.600	0.0224	0.0044919	
21	0.8	29.3	23.440	492.240	0.0183	0.0036604	
22	0.8	31.3	25.040	550.880	0.0153	0.0030574	
23	0.8	25.4	20.320	467.360	0.0127	0.0025538	
24	0.8	25.4	20.320	487.680	0.0106	0.0021331	
25	0.8	24.3	19.440	486.000	0.0089	0.0017817	
26	0.8	21.4	17.120	445.120	0.0074	0.0014882	
27	0.7	15.2	10.640	287.280	0.0054	0.0010877	
28	0.6	14.3	8.580	240.240	0.0039	0.0007787	
29	0.54	14.3	7.722	223.938	0.0029	0.0005854	
30	0.42	10.2	4.284	128.520	0.0019	0.0003803	
31	0.38	9.2	3.496	108.376	0.0014	0.0002874	
32	0	0	0.000	0.000	0.0000	0.0000000	

In the present study percent mortality of egg stage ranged from 7 to 8. Even though the life expectancy of *M. incognita* infected with *P. penetrans* extended by 2 days, mortality rate of J2 was high (19 %) (Table 2) when compared to healthy J2 (7 %) (Table 3). These results were in accordance with Bansa – Singh and Dhawan (1994) who showed negative correlation between the number of

spores per J2 and penetration of J2 into the roots. Encumbered spores reduced the mortality of J2 and maintained them in active state which provides them a greater chance in contacting the host (Davies *et al.*, 1991). The duration from J2 to J3 was increased by one day. Mortality rate of *P. penetrans* infected J3 was high (9 %) when compared to healthy population (1 %) (Table 2 & 3). The mortality

of healthy J2 was less after reaching the host. This could be due to stable environment compared to complex soil ecosystem where various factors affect the J2 (Singh and Sharma 1994). From J3 to J4 no mortality was recorded. The duration of *P. penetrans* infected and healthy adult stage remained the same. *P. penetrans* infection extends the life cycle of *M. incognita* by 3 days. Survival and life expectancy of *M. incognita* parasitized by *P. penetrans* was reduced by 2 days compared to healthy population.

**Stable age distribution of healthy and *Pasteuria penetrans* infected *M. incognita***

The contribution made by different

developmental stages was determined by analyzing the stable age distribution. The study on stable age distribution revealed that per cent distribution of various life stages was higher in healthy *M. incognita* and lowest in *M. incognita* parasitized by *P. penetrans*. The distribution of encumbered population of J2 was reduced to nearly 41 per cent (Table 4) of the healthy population (79 %) (Table 5). The distribution of J2 was much affected by *P. penetrans* when compared to other stages. Rangaswamy *et al.*, (2001) reported that the development of J2 into adult was lower in *P. penetrans* infected nematodes. The per cent survival of infected female decreases with increase in time spent by the J2 in soil (Giannakou and Gowen, 2004).

**Table.8** Population growth statistics of *M. incognita* infected with *P. penetrans*

Parameters	<i>M. incognita</i> infected with <i>P. penetrans</i>
Gross reproductive rate ( $\sum m_x$ )	80.3
Net reproductive rate ( $R_0 = \sum l_x m_x$ )	39.16
Mean length of generation (days) $\{T_c = (\sum l_x m_x) / R_0\}$	25.09
Innate capacity for increase (females/female/day) $r_m = (\log R_0) / T_c$	0.14
Finite rate of increase in number (females / females / day) ( $\Lambda = e^{r_m}$ )	1.15
Rate of weekly multiplication of population ( $e^{r_m}$ ) <sup>7</sup>	2.74
Doubling time ( $\text{Log}_2 / \log \lambda$ )	4.80

**Table.9** Population growth statistics of healthy *M. incognita*

Parameters	<i>M. incognita</i>
Gross reproductive rate ( $\sum m_x$ )	292.1
Net reproductive rate ( $R_0 = \sum l_x m_x$ )	219.27
Mean length of generation (days) $\{T_c = (\sum l_x m_x) / R_0\}$	22.98
Innate capacity for increase (females/female/day) $r_m = (\log R_0) / T_c$	0.23
Finite rate of increase in number (females / females / day) ( $\Lambda = e^{r_m}$ )	1.26
Rate of weekly multiplication of population ( $e^{r_m}$ ) <sup>7</sup>	5.16
Doubling time ( $\text{Log}_2 / \log \lambda$ )	2.95



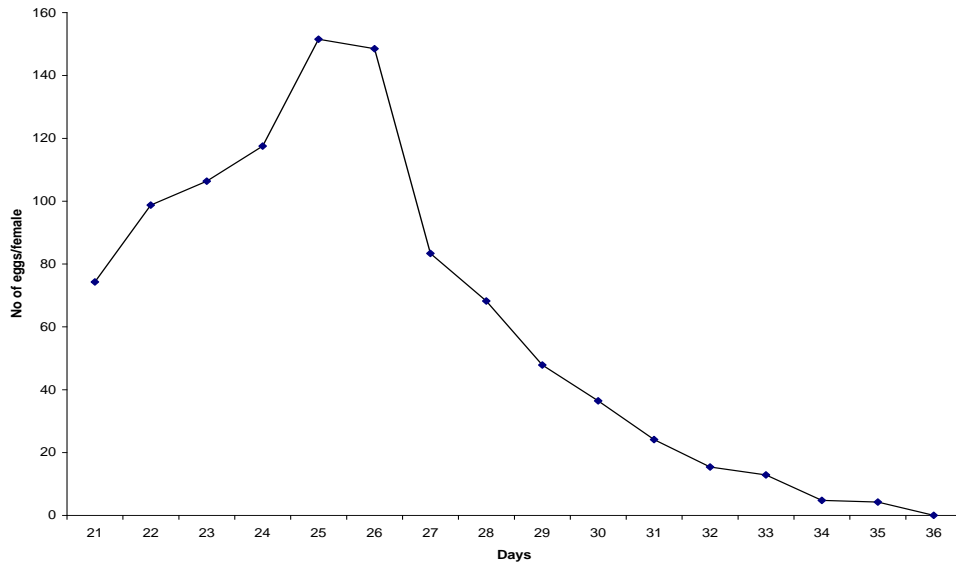


Fig.1 Age specific fecundity for females infected with *P. penetrans*

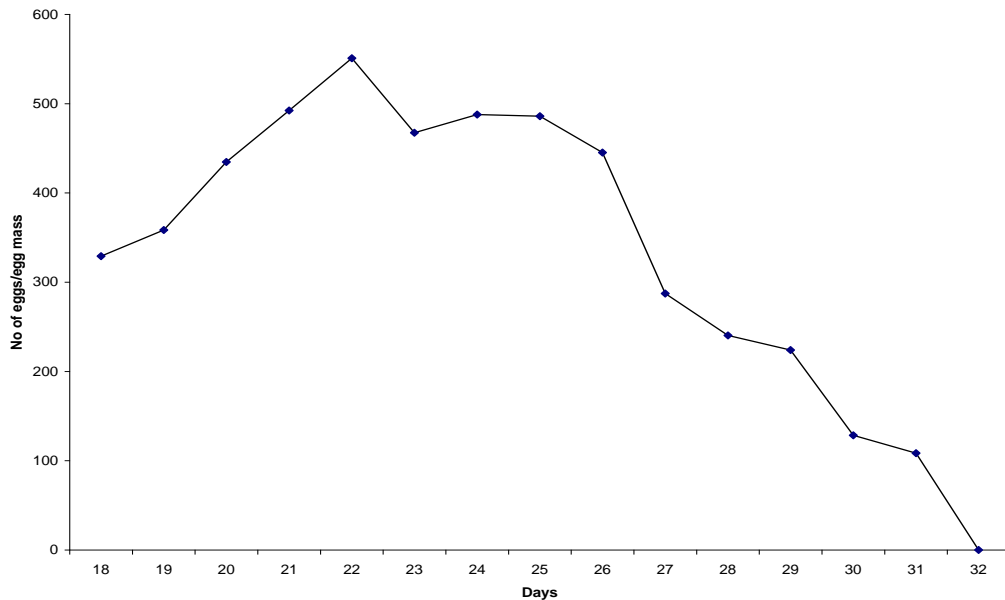


Fig.2 Age specific fecundity for healthy females

**Population growth statistics of healthy and *Pasteuria penetrans* infected *M. incognita***

The study revealed that egg laying of healthy female starts from 18<sup>th</sup> day but in *P. penetrans* infected *M. incognita* egg laying starts from 21<sup>st</sup> day (Fig 1 and Fig 2). The fecundity of the *P. penetrans* infected females was very

low. Some spores of *P. penetrans* attached to *M. incognita* juveniles failed to germinate. These juveniles produced eggs. The egg laying capacity of these females were partially affected (Fig 1). This was in accordance with Pembroke *et al.*, (2004) who reported that some encumbered J2 which does not show spore germination has laid few eggs.

Eggs per egg mass ranged from 90-148. Average eggs laid by the infected females were 80.3 eggs per female. The fecundity was reduced by 4 -5 times when compared to healthy *M. incognita*. This shows the parasite is highly efficient in arresting the fecundity of *M. incognita* significantly.

The decrease in innate capacity of *P. penetrans* infected root knot nematodes showed that the nematodes lost its ability for efficient multiplication compared to healthy nematodes. This leads to subsequent increase in doubling time of *M. incognita* infected by the *P. penetrans*. The rate of weekly multiplication of *M. incognita* population was lowered by 2.42 times (Table 8 and 9). The females developing inside the root was reduced significantly due to the spore burden on them (Davies *et al.*, 1991).

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