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Population parameters and life fertility table of root-knot nematode (*Meloidogyne incognita*) on cauliflower (*Brassica oleracea* var. *botrytis*)

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Abstract
The study was conducted under growth chamber at the Department of Entomology, College of Horticulture, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2015-16 to developed the life fertility table of *M. incognita* on cauliflower. A life fertility table for *Meloidogyne incognita* was constructed on cauliflower cv. Pusa Snow K-1 at 25±1ºC. Mortality rates of *M. incognita M. incognita* life stages were very high during egg and J_2 stages prior to root penetration. Mortality of subsequent life stages was low and virtually constant. The gross reproductive rate was 348.69 eggs/ female, whereas net reproductive rate was 62.65 eggs/ female. The approximate generation time taken by the nematode at constant temperature was 21.61 days. The values for intrinsic capacity for natural increase and true intrinsic rate of increase were 0.19 and 0.16. The initial days of egg laying contributed more to the value of r_m than other age intervals. The nematode took 21.44 days to complete its one generation at respective temperatures. The value for finite rate of natural increase was 1.65 (25±1°C). The time taken by the population to double itself was 3.59 days.

Keywords: *M. incognita*, gross reproductive rate, net reproductive rate, approximate generation time, true intrinsic rate of increase, finite rate of natural increase

1. Introduction
Root knot nematodes are a serious biotic production constraint affecting vegetable production in India. The root-knot nematode, *Meloidogyne incognita* Kofoid and White (Chitwood), is widely distributed in the major vegetable producing states of India. Predictions of nematode population levels are important for making management decisions. Life table study is very useful to analyse the mortality of nematode population and to determine key factors responsible for the highest mortality within population.

A life table is a tabular device which describes for every particular age of interval. In ecological study life table is an important analytical tool, which provides detailed information of population dynamics to generate simple but more informative statistics. It also gives a comprehensive description of the survivorship, development and expectation of life (Yzdani and Samih, 2012) [14]. Life tables may be used to study inherent differences in the survivorship and reproductive strategies of populations under different ecological regimes (Afrane *et al*., 2007) [1]. Life table, studies provide an opportunity to assess and evaluate the impact of specific mortality factors acting on insect population (Harcourt, 1969; Mohapatra, 2007) [8]. It can also give information about the changes which occur during the developmental stages and their relationship with different environmental factors (Atwal and Bain, 1974) [2]. Life tables constructed using laboratory data collected under controlled conditions and are useful in revealing the maximal growth potential of a population (Gabre *et al*, 2004) [7]. Life tables used can make quantitatively and qualitatively evaluation of various host plants.

2. Materials and Methods
2.1 Root-knot nematode culture
Single egg mass of *M. incognita* was isolated from tomato roots collected from Entomology farm of University (UHF, Nauni-Solan). This was placed singly in Petri plate containing distilled water. The second stage juveniles hatched from single egg mass were inoculated on root of brinjal (var. Pusa Purple Long) seedling grown under aseptic conditions in 500g soil capacity plastic pot. After 45 days of inoculation, the plant was uprooted and egg masses were isolated from the roots with the help of a forecep.

The egg masses were placed in petri plate containing distilled water. The eggs were allow to hatch and the juveniles were again inoculated in the brinjal seedlings (5 seedlings) grown individually in 500 cc capacity plastic pots (containing autoclaved soil and sand mixture 1:1 w/w) under greenhouse conditions. The seedlings were allowed to grow for 60 days under aseptic conditions. This nematode culture was used further for mass multiplication

For mass multiplication, seedlings of brinjal were raised in thirty plastic pots of one kg capacity and maintained under aseptic conditions. After one week the seedlings were inoculated with freshly 500 juveniles of *M. incognita* by making 4-5 holes around the stem (Campos and Campos, 2005) [5]. The seedlings were allowed to grow and maintained till the culture was required for the experimentation.

2.2 Identification of the nematode species

The character most frequently used for identification of *Meloidogyne* at species level is the morphology of finger print like perineal pattern, specific of the species, observed in the posterior body region of adult females. This area comprises the vulva-anus area (perineum), tail terminus, phasmids, lateral lines and surrounding cuticular striations (Siddiqi, 2000) [11]. Perineal patterns of individual female of *Meloidogyne* were cut and prepared as per the methods given by Karssen (2002) [9].

Fresh galled roots were selected from uprooted pot culture plants to isolate mature females. The galled roots were stained with acid fuchsin/cotton blue-lactophenol method given by Bridge *et al*., (1980) [4]. The roots were preserved in clean lactophenol. The galls were opened by teasing them carefully with fine needle to expose the root-knot nematode female. The females were taken out and prepared the perineal pattern slides of individual female as per the method given by Karseen (2002) [9].

2.3 Raising of seedlings

Seedlings of cauliflower cv. Pusa Snow K-1were raised in 3 Kg capacity sterilized plastic pots containing autoclaved soil mixture (soil, sand and FYM in the ratio of 2:1:1 respectively). These pots were kept in glasshouse for germination of the seedlings.

2.4 Preparation of soil mixture

The soil mixture (soil, sand and FYM in the ratio of 2:1:1) was autoclaved in gunny bags at a temperature of 121°C (pressure 15 lbs/ sq inch) for one hour. After autoclaving, the mixture was spread over the polythene sheets for 24 hours to make it free from toxic fumes.

2.5 Isolation/ hatching of egg masses

Egg masses were isolated from the roots of brinjal plants (maintained for the nematode culture) and juveniles of an individual egg mass was kept in a cavity block with distilled water. For life table study of each crop 200 cavity blocks were maintained. These were incubated at 25°C for egg hatching. Juveniles emerged in each cavity block were counted and removed daily for up to 7 days of incubation. The percentage of egg hatching was calculated accordingly.

2.6 Inoculation of juveniles (J_2s)

Cauliflower seedlings were uprooted carefully from the nursery pots without damaging the roots and were transplanted in small (50g capacity) plastic cups @ single seedling per cup. About 200 seedlings of each crop were maintained separately for two different experiments, conducted at constant temperature (25±1°C). After one week of transplanting each seedling was inoculated with freshly hatched juveniles extracted from individual egg mass (@. 350).

2.7 Juvenile penetration and development

Five inoculated seedlings were uprooted after every 24 hours to check the penetration of juveniles entered in the roots. All juveniles that had entered the roots within the same time constituted an inoculums cohort of the same age.

2.8 Staining of roots

To study each development stage and age specific survival, five seedlings were removed daily and washed free of water. The clean roots were stained with 0.1 per cent cotton blue lactophenol solution and stored in clear lactophenol to remove the excess stain. Penetration was checked by compressing the stained roots between two glass plates (15 × 10 cm) under stereo zoom microscope.

2.9 Observations recorded:

Number and different stages of nematode (J_2, J_3, J_4 and adult) were identified under the microscope. The J_2s were identified by the presence of stylet (infective and feeding stage) whereas J_3 and J_4 were non-feeding stages having a spike tail stage. The males and female stages were identified by the presence of different genital primordias. The males of root-knot nematode having 'I-shaped' genital primordia while females having 'V-shaped' genital primordial. The sex ratio was determined at J_4 stage.

2.10 Age specific survival and fecundity of females

From 22 days onwards number of females, males and eggs per egg mass were calculated individually. Ten egg masses were collected and the number of egg per egg mass was counted daily to estimate number of eggs laid /female/day separately. The females were assumed dead as soon as the egg laying was stopped. However females were checked for egg laying even for one week.

2.11 Construction of life table

The life table of *M. incognita* was studied under laboratory conditions on cauliflower (Pusa Snow K-1) at room temperature. The fertility tables were constructed as per the method described by Singh and Sharma, 1995 [12].

2.12 Age specific survival/mortality life table

The age specific survival/mortality life table was constructed as described by Deevey, 1947 [6]:

x= age of cohort

l_x= number surviving at the beginning of age x age of individual in days (pivotal age).

L_x = proportion of individuals still alive at age x (age specific survival), l_x for females was calculated from l_x for immature and for adult stages.

m_x = mean of female off spring produced per female in the age interval (x). The daily increase in egg number per female was taken as age specific fecundity.

The following fertility parameters were calculated:

Gross reproduction rate (GRR): $\sum m_x$

Net reproductive rate (R_o): $\sum l_x m_x$
Approximate cohort generation time (T_c): $\sum x l_x m_x / R_o$

Innate capacity for natural increase (r_c): $\log_e R_o / T_c$

True intrinsic rate of increase (r_m): The true intrinsic rate of increase was calculated as per the method of Southwood, 1978 [13] was used of r_m.

True generation time (T): loge Ro / r_m.

2.13 Statistical Analysis: The experiment was conducted under laboratory condition using completely randomized design (CRD) and the data obtained were analysed in MS-Excel 2007 and presented in the form of tables and graphs.

3. Results

3.1 Survival and life expectancy (e_x) of *Meloidogyne incognita* on at room temperature

For evaluation of survival and life expectancy (e_x) of *M. incognita* on cauliflower cv. Pusa Snow K-1**,** the mortality rate on days 0 and 1 were found very high (177 and 74 respectively) in comparison to days 2 to 23 whereas, it varied between 1-4 with increasing or decreasing trend irrespective of age interval. Although, survivorship (l_x) remained highest during 0 and 1 day age interval (350 and 173, respectively) it was found abruptly reduced on day 3 (99) and thereafter reduced gradually (99-60) till 24th day and remained same on 25th day also (60). Life expectancy increased from 0-2 days age interval (6.31-18.29) and thereafter showed gradual decline till 24th day (17.47-1.50) and was recorded minimum on 25th day (0.50).

Table 1: Survival and life expectancy (e_x) of *M. incognita* on cauliflower at constant temperature (25±1°C).

x	l_x	d_x	100qx	L_x	T_x	e_x
0	350	177	50.57	261.5	2208	6.31
1	173	74	42.77	136	1946.5	11.25
2	99	1	1.01	98.5	1810.5	18.29
3	98	2	2.04	97	1712	17.47
4	96	1	1.04	95.5	1615	16.82
5	95	3	3.16	93.5	1519.5	15.99
6	92	3	3.26	90.5	1426	15.50
7	89	2	2.25	88	1335.5	15.01
8	87	4	4.60	85	1247.5	14.34
9	83	1	1.20	82.5	1162.5	14.01
10	82	1	1.22	81.5	1080	13.17
11	81	1	1.23	80.5	998.5	12.33
12	80	2	2.50	79	918	11.48
13	78	1	1.28	77.5	839	10.76
14	77	3	3.90	75.5	761.5	9.89
15	74	2	2.70	73	686	9.27
16	72	2	2.78	71	613	8.51
17	70	1	1.43	69.5	542	7.74
18	69	3	4.35	67.5	472.5	6.85
19	66	1	1.52	65.5	405	6.14
20	65	1	1.54	64.5	339.5	5.22
21	64	2	3.13	63	275	4.30
22	62	1	1.61	61.5	212	3.42
23	61	1	1.64	60.5	150.5	2.47
24	60	0	0.00	60	90	1.50
25	60	60	100.00	30	30	0.50

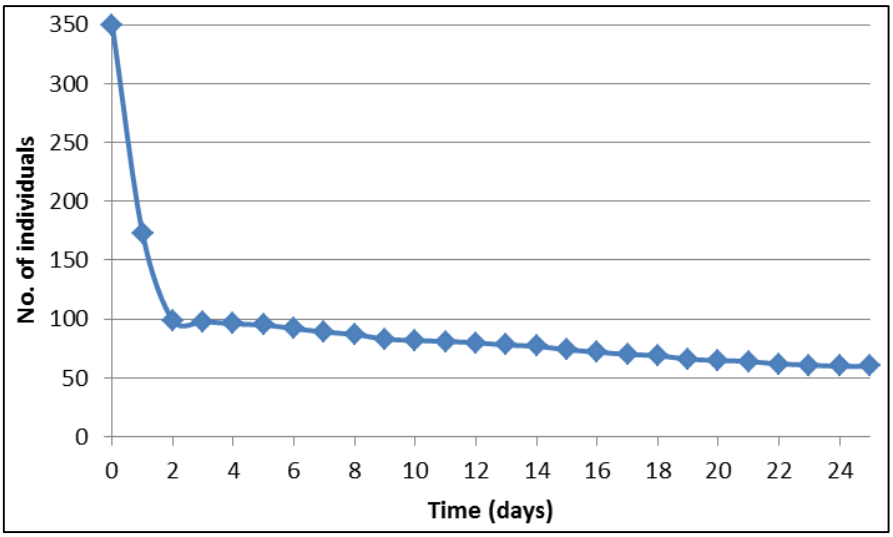

Fig 6: Survival curve for *M. incognita* on cauliflower (25±1°C)

3.2 Age-specific survival and age-specific fecundity of *M. incognita* at (25±1°C).

The adults emerged for the first time on 20 day of the pivotal age with a survival rate of 19%. This rate of survival kept on decreasing by one per cent after every two days and attained a value of 17% on 24 day of the pivotal age.

The first egg laying was observed on 20 day of the pivotal age (73.96 eggs/ female) which increased to 111.69 eggs/ female on 21 day of the pivotal age. This value was the peak egg laying recorded so far in the experiment and thereafter, reduced abruptly as the pivotal age increased. Least numbers of eggs were laid on 24 day with the value of 39.02 eggs/ female after which the egg laying was completely stopped on 25 day of the pivotal age.

Table 2: Age-specific survival and age-specific fecundity of *M. incognita* on cauliflower at 25±1°C.

Pivotal age in days (x)	Survival at x (l_x)	Number of eggs/female (m_x)	l_xm_x	xl_xm_x	Trial r_m $e^{7-rmx}l_xm_x$	
					r_m=0.19	r_m=0.20
0-19		Immature stages				
20	0.19	73.96	13.74	274.72	337.08	275.98
21	0.18	111.69	20.42	428.87	414.27	335.80
22	0.18	66.45	11.77	258.95	197.46	158.47
23	0.17	57.57	10.03	230.77	139.15	110.56
24	0.17	39.02	6.69	160.55	76.75	60.38
25	0.00	0.00	0.00	0.00	0.00	0.00
Total	-	348.69	62.65	1353.86	1164.72	941.18

3.3: Population growth parameters of *M. incognita* on cauliflower (cv. Pusa Snow K-1)

The fertility parameters of *M. incognita* on cauliflower cv. Pusa Snow K-1 at constant temperature (25±1°C), the gross reproductive rate was 348.69 eggs/ female, whereas net reproductive rate was 62.65 eggs/ female. The approximate generation time taken by the nematode at constant temperature was 21.61. The values for intrinsic capacity for natural increase and true intrinsic rate of increase were 0.19 and 0.16, respectively. The initial days of egg laying contributed more to the value of r_m than other age intervals (Tables 4 & 5). The nematode took 21.44 days to complete its one generation at respective temperature. The value for finite rate of natural increase were 1.65 (25±1°C). The time taken by the population to double itself was 3.59 days.

Table 3: Fertility parameters of *M. incognita* on cauliflower.

Fertility parameters	25±1°C
Gross reproductive rate (GRR)	348.69
Net reproductive rate (R_o)	62.65
Approximate generation time (T_c)	21.61
Innate capacity for natural increase (r_c)	0.190
True intrinsic rate of increase (r_m)	0.193
True generation time (T)	21.44
Finite rate of natural increase (λ)	1.65
Doubling time (DT)	3.59

Table 4: Contribution of each age group to the value of r_m (r_m =0.193) in cauliflower at 25±1^{0}C.

Pivotal age group (x)	$l_xm_x.e^{7-rmx}$	Percent contribution of each age group
20	317.45	29.06
21	388.97	35.60
22	184.85	16.92
23	129.88	11.89
24	71.42	6.54

4. Discussion

The life table study of *M. incognita* indicated that age had a profound impact on the survivorship of larval population. This trend indicates the delicacy of the earlier instars as compared to the later instars (Singh and Sharma, 1995; Banu *et al*., 2003) [12]. Reduced survivorship in early days indicates high larval mortality which is an important check to counterbalance the effect of reproduction in mature stage. The mortality prior to attain the sexual maturity plays a key role in decreasing the reproduction of nematode. Cohort life table is suitable to study the dynamics of nematode population because it helps to estimate the parameters related to growth potential. After first three juvenile stages of the nematode was best able to survive and reach to maturity with zero mortality. Furthermore the more delicate age in the insect life confined to initial days which can be used as weak link to manage the pest effectively.

5. Conclusion

The maximum mortality was recorded at initial stage only, which may be due to egg/juvenile mortality and inability of second stage juvenile to penetrate the roots (Singh and Sharma, 1995) [12]. The low mortality inside the root may be attributed to the availability of food and optimum environmental conditions for survival and multiplication. The intrinsic rate of increase (r_m) has been useful as predictive and comparative measure of population growth potential. In the present study, initial egg laying contributed greatly to the value of r_m which was in accordance with the findings of Singh and Sharma, 1995 [12] for *Heterodera cajani* and Banu *et al*., 2003 [3] for *M. incognita*.

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