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Variation in Life Cycle of *Meloidogyne Incognita* in Different Months in Indian Condition

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Abstract: ***The life cycle of M. incognita in tomato and brinjal seedlings during different months at different temperature was recorded. The life cycle of M. incognita seems to be related with temperature. The most ideal temperature for the development of M. incognita seems to be between 20-25^0 C. It was observed that inoculated seedlings raised during the month of June showed 2nd stage juveniles started invading within 40- 48 hours after inoculation. Maximum penetration of J_2 was observed on the 5th day after inoculation. The life cycle of the nematode was completed within 16 days during the month of June i.e. from 2nd stage juveniles to 2nd stage juveniles. The life cycle studies in June, July and August also showed similar trend whereas in September it was reduced by one day and in October it was extended by two days. During the month of November the life cycle was completed in 25 days for completion of one generation. During the month of December and January although seedlings were infected by second stage juveniles but further no development occurred due to extreme low temperature.***

Keywords: *M. incognita*, life cycle, tomato and brinjal

1. Introduction

Root-knot nematode damage results in poor growth, a decline in quality and yield of the crop and reduced resistance to other stresses (e.g. drought, other diseases). A high level of root-knot nematode damage can lead to total crop loss. The infection starts when infective second-stage juveniles (J_2) hatch from eggs in the soil and penetrate plant roots behind the root cap and migrate intercellularly in the cortical tissue to the vascular cylinder and then become sedentary. Then they inject secretions into five to seven undifferentiated procambial cells in the vascular cylinder near the head of the J2 to become multi-nucleate (Hussey and Grundler, 1998) and to form very specialized feeding cells called giant cells, on which the juveniles (J_2) and later the spherical females feed (Sijmons*et al.,* 1994). Furthermore, cell size increases dramatically, walls are remodelled by formation of ingrowths and the cytoplasm becomes dense with an increase in cell organelles (Baum *et al.*, 1996). With nourishment from the giant-cells, the root-knot nematodes complete their life cycle. Then females lay eggs into a gelatinous matrix outside the roots surfaces. The infection of the root systems by phytoparasitic nematodes of the genus *Meloidogyne*is easily recognized by one formation of galls. These galls descend from the development of the giant cells and the females inside the roots. Nematode damaged roots do not utilise water and fertilisers effectively, leading to additional losses. Infection of young plants may be lethal, while infection of mature plants causes decreased yield. *Meloidogyne*species constitute the major nematode problem in developing countries. Nematode reproduction is very fast. On average, a typical life cycle may be only 17 days at summer temperatures. This means that even if nematode numbers are low at the beginning of the growing season, nematode populations can rapidly increase and can become harmful to the crop in a relatively short period of time. These nematodes infect nearly all major crops and are therefore widespread. Damage can be directly observed by examining the roots, because root-knot nematodes produce galls or knot-like swellings along the plant roots. Therefore objective was taken so as to study the life cycle in different month so as to adapt proper control measure for the management of root-knot disease.

2. Materials and Methods

The experiment was conducted in small ice cream cups (6.5 cm) containing 50 gm of sterilized soil. Thirty cups were used as replicates per test crop. Ten seeds of each crop were seeded in each cup and were watered to provide enough moisture to germinate the seeds. After germination of seeds (3-4 leaf stage) the known population (5000 active juveniles) of *M. incognita* was inoculated in each cup uniformly. The cups were watered at regular interval to maintain the moisture level.

Root system of 10 inoculated seedlings of each crop were taken out daily, thoroughly washed and stained with boiling 0.1% (w/v) acid fuchsin in lactic acid, glycerol and distilled water (1:1:1) as per method described by Bridge *et al.*, (1981). The stained roots of the seedlings were then transferred into a Petri dish and the root-knots were cut and separated from the root system. For detection of the developing nematodes, each root-knot was transferred onto a slide containing few drops of glycerin. After teasing root knots, the developing stages were separated from the debris and slides were prepared for identification of stages. Further microscopic photographs were taken. The observations were continued till J_2 were observed. For studying the life cycle of *M. incognita* in different months, Tomato was seeded in ice cream cups in different months viz., November, December, January, and February and Brinjal was seeded in the month of June, July, August, September and October and inoculated with 5000 active juveniles of *M. incognita*. The seedlings were taken out daily and the observations were made daily till the J_2 were released from freshly laid eggs and different stages of *M. incognita* were photographed.

3. Results and Discussion

The data on life cycle of *M. incognita* in tomato and brinjal seedlings during different months are presented in Table 1. Daily observations of the inoculated seedlings raised during the month of June showed 2nd stage juveniles started invading within 40- 48 hours after inoculation. Maximum penetration of J_2 was observed on the 5th day after inoculation. Subsequently development of 3rd and 4th stages after second moulting was completed by 5 and 7 days after inoculation and after third moulting adult females was observed on 9th day. The first egg laying was observed on 11- 12th day and second stage larvae were released on 16th day after inoculation. Thus the life cycle of the nematode was completed within 16 days during the month of June i.e. from 2nd stage juveniles to 2nd stage juveniles (Plate 1).

The life cycle studies in June, July and August also showed similar trend whereas in September it was reduced by one day and in October it was extended by two days. Another important feature noticed was relatively larger size of root galls with higher number of eggs and juveniles per root gall during August and September. The number of eggs or juveniles per root gall ranged between 450 and 15000 during these months.

The life cycle of this nematode during different month of sowing and inoculation indicated increase in the period of life cycle. During the month of November the life cycle was completed in 25 days for completion of one generation. During the month of December and January although seedlings were infected by second stage juveniles but further no development occurred due to extreme low temperature. The second moult that occurs very often on 5-7 day during June-July and 3rd stage remains for 4 days when third moult occurred resulting into 4th stage male and female juveniles. Vulvur plates in the fourth moultwere observed on 11-13th day. Deposition of gelatinous matrix of egg masses was observed on 12-13th day. Second stages J_2 were observed on 15-16th days in the month of June. During December and January second moulting was recorded 27 days of inoculation. In most of the cases the J_3 did not grow and perhaps degenerated, however J_4 could be observed between 45-50 days after inoculation. Only few adults without eggs could be observed between 50-55 days of inoculation. From this observation it is calculated that during the month of December even if there is infection, the nematode may not affect the crop due to extremely poor development. It also indicates that in December- January, biomass of the nematode may never reach to a level that would bring out pathogenic effect on the plant.

4. Reproductive Biology

The reproduction of nematode *M. incognita* was greatly influenced by temperature in different months (Table 2). During the month of June number of eggs and females in a knot ranged between 100-150 and 1-7 respectively, while in the month of July the number of eggs and females ranged between 250-500 and 1-15 per knot respectively in 30 day old seedlings. However, during August the number of eggs ranged from 450 to 2000 per root knot. This population increased in the month of September and a range of 500 to 15000 eggs and 1-65 females were recorded from a knot. In the month of October the number did not increase and it was found in the range of 250-12000 per knot. During the month of November 50-150 eggs were recorded in 30 days old tomato seedlings; however, in the month of December and January, there was no reproduction. With the commencement of February due to rise in temperature 50-250 eggs and 1-7 females were produced.

Bridge and Page (1982) have reported the similar observations on the life cycle of *M. graminicola*. However, Rao and Israel (1973) have reported longer span of life cycle in different months. They reported that 3rd stage appeared on day 6 and the 4th stage was seen on day 9. Males were first observed on day 24 and females with ovisac on day 27. This indicates that there have been some lapses in observations. Dabur*et al.*, (2004) has also reported that *M. graminicola* required 24 days for completion of one life cycle. They reported that J_2, J_3 as well as J_4 were seen in the roots on 8 day after sowing. They further stated that on day 10 adult male and females were recorded in the roots. They, however,recorded egg laying 20 days after sowing. This indicates that adult females required additional 10 days for egg laying, which seems to be incorrect. They conducted experiment in the month of July which is more or less similar to eastern U.P. Jaiswal and Singh (2010) reported that life cycle of *M. graminicola* was completed in 26-51 days during different months. The difference in the life cycle may be attributed to the methods used and environmental condition of their working sites. For life cycle studies these workers used either infested soil or eggs as inoculums, which give staggered infection.

The life cycle of *M. incognita* seems to be related with temperature. The most ideal temperature for the development of *M. incognita* seems to be between 20-25^0 C. However, when the temperature decreases development of the nematodes is delayed. It is only because of the decrease in temperature the life cycle is completed in 25 days in November, whereas in December it takes more than 50 days. The fecundity of this nematode also varies with the availability of nutrients and temperature. Extremely low temperature reduces the fecundity rate of this nematode.

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Table 1: Life cycle of *M. incognita* during different months in Tomato and Brinjal

Month	*Crop*	*Variety*	*Period*
June 2008	Brinjal	Pusa Purple	16 days
July 2008	Brinjal	Pusa Purple	16 days
August 2008	Brinjal	Pusa Purple	16 days
September 2008	Brinjal	Pusa Purple	15 days
October 2008	Brinjal	Pusa Purple	17 days
November 2008	Tomato	Pusa Ruby	25 days
December 2008	Tomato	Pusa Ruby	*
January 2009	Tomato	Pusa Ruby	*
February 2009	Tomato	Pusa Ruby	27 days

* Penetration occurred but no further development occurred.

Table 2: Effect of different temperature in different months on the Reproductive biology of *Meloidogyne incognita*

Host	*Month*	*No. of eggs/root-knot (gall)*	*No. of females/root-knot (gall)*
Brinjal	June	100-150	1-7
Brinjal	July	250-500	1-15
Brinjal	August	450-2000	1-28
Brinjal	September	500-15000	1-65
Brinjal	October	250-12000	2-50
Tomato	November	50-150	1-3
Tomato	December	-	-
Tomato	January	-	-
Tomato	February	50-250	1-7

Plate 1.Life cycle of *Meloidogyne incognita*

1. Second stage juveniles penetrate plant roots behind the root cap.
2. Second stage juveniles migrate intercellularly in the cortical tissue.
3. Formation of first root-knot in tomato seedling.

A. Second stage juvenile of *Meloidogyne incognita*.
B. Developing second stage juvenile*Meloidogyne incognita*.
C. Developing second stage juvenile*Meloidogyne incognita* with “I” shaped genital primordium.
D. Third stage of *Meloidogyne incognita*
E. Fourth stage of *Meloidogyne incognita*
F. Adult females of *Meloidogyne incognita*
G. Egg masses in gelatinous matrix of *Meloidogyne incognita*

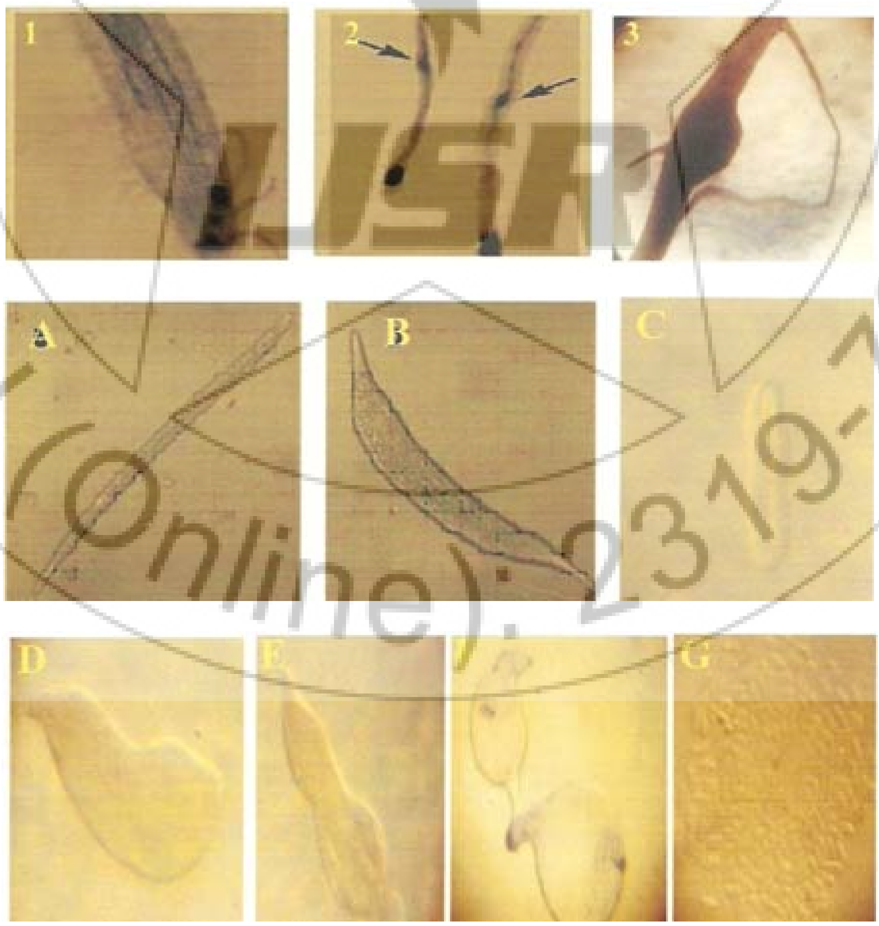

Plate 1: Life cycle of*Meloidogyne incognita*