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Effect of different temperature on the life cycle of root-knot nematode, *Meloidogyne incognita* on okra

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Abstract
The life cycle and development of root-knot nematode, *Meloidogyne incognita*, was studied in the roots of okra in a screen house for comparison. Length of the life cycle in root-knot nematodes is greatly influenced by temperature. The j_2 of *M. incognita* penetrated into okra roots after 48 h in the month of April-May while in the month of March-April in 72 h. The third stage (j_3) appeared on 8 DAI in the month of April-May as compared to month of March-April in 10 DAI. Young females were first noticed on 22 DAI and 24 DAI for the month of April-May and March-April, respectively. The life cycle of the root-knot nematode, *M. incognita* from j_2-j_2 stage was completed in 33 days in the month of March-April and 29 days in the month of April-May under screen house conditions.

Keywords: Root-knot nematode, *Meloidogyne incognita*, okra, temperature, life cycle, development

Introduction
Okra is one of the warm season crops grown in the tropical and sub-tropical regions of the world [6]. As more intensive and continuous cultivation increased, soil-borne diseases and including plant-parasitic nematodes (PPNs) in vegetable production. PPNs are considered a major unmanageable biotic cause of plant stress and crop loss. PPNs are capable of producing recognizable disease symptoms on suitable susceptible hosts. It is known to be highly susceptible to root-knot nematodes and infected plants are stunted, exhibiting signs of nutrient deficiency and characteristics large irregular swellings on both primary and secondary roots [8]. This nematode has a wide host range with okra being a major economically important host. However, the yield loss may be as high as 60 per cent in continuously cultivated crop of vegetables due to root-knot nematode [5]. Estimated over all annual yield losses due to *M. incognita* in okra has been recorded more than 14 per cent [2] Damage caused by the root knot nematode is determined by the initial nematode density which effect and yield of annual crops. The minimal density that causes a measurable reduction in plant growth or yield varies with nematode species, host plants, cultivar and environment [1]. Most PPNs of okra complete their life cycles (egg, four pre-adult juvenile stages, egg-producing adult) in two to four weeks depending on the nematode species and environmental conditions.
Although much is known about the life cycle duration of *M. incognita*, less is known about the influence of different temperature on this nematode's life cycle. With changing weather patterns, in-depth knowledge about the effect of temperature on the life cycle of *M. incognita* is becoming increasingly important. Therefore, a pot study was undertaken to effect different temperature on life cycle studies of *M. incognita* on okra under screen house condition.

Materials and Methods
Present study was carried out to determine the life cycle of root- knot nematode, *M. incognita* in okra crop under screen house of the Department of Nematology, CCS Haryana Agricultural University, Hisar. Root knot nematode, *M. incognita* was maintained in okra plants in the pots filled in the infested soil inside the screen house and from the field infected plants were also uprooted, carefully washed in running tap water and egg- masses and j_2 larvae were collected in to Petri dishes containing distilled water and inoculated to the pots for mass culturing of *M. incognita.*
For life cycle experiment, pots were filled with steam sterilized sandy loam soil autoclaved at 121±1 ^{0}C temperature and 15 lbs pressure.

The seeds were allowed to germinate and thinned to one seedling/pot (1 kg soil capacity) followed by inoculation of j_2 of *M. incognita* @ 1000j_2/kg soil at 7 days after sowing. The roots were covered with the soil inside the pots immediately after inoculation. Each treatment was replicated three times with completely randomized design. Regular watering was done according to the crop requirement and from 24 hours onwards after inoculation, started taking the observations. The plants were uprooted according to the treatment schedule: Time taken for penetration, observations were recorded daily upto 3 days and subsequently on alternate day's upto 15 days, time taken to complete different developmental stage of the nematode, i.e., j_2-j_3, j_3-j_4 and j_4-adults and time taken to complete the life cycle (j_2- j_2). At each interval, three plants were uprooted without any damage to roots system and brought to the laboratory. These roots were washed gently to remove adhering, soil particles. The roots were stained in 0.1% acid fuchsin lectophenol. After that, roots were again washed under running water followed by placing the roots in plain lectophenol for destaining for 24 hours. Roots were observed under stereoscopic microscope for teasing out the different developmental stage.

Result and Discussion

Life cycle of root-knot nematode, *M. incognita* on okra in the month of March-April

The life cycle of root-knot nematode, *M. incognita* in okra was studied under screen house conditions. The second stage active juveniles (j_2) of *M. incognita* penetrated into young roots of okra plant near the root tip within 72 hours (h). However, the visible swelling on the infected root was observed on 5th day after inoculation (DAI). On the 5th day after inoculation, j_2 slightly enlarged in size (Table 1). Some of the j_2 were seen in second moulting stage. Several j_2 invaded the same root inducing 1-10 galls per plant. The j_2 that had penetrated into the roots developed into third-stage juveniles (j_3) on 10 DAI which was developed into fourth-stage juveniles (j_4) at 19 DAI. Young females were observed on 24and males on 26 DAI inside the root. Males in the soil were observed on 29 DAI. Egg deposition started at 30 DAI inside the root. On 33 DAI, six j_2 of the second generation were recorded inside the root. Thus, life cycle of the root-knot nematode, *M. incognita* from j_2-j_2 stage was completed in 33 days under screen house conditions in the month of March-April. Some of the second generation larvae (j_2) developed further inside the old galls while others invaded new roots and induced new galls at root tips.

Table 1: Time taken for life cycle of root-knot nematode, *M. incognita* on okra in the month of March-April

Date	Observations (Days after inoculation)	Developmental stage of nematode	Remarks	Temperature (^{0}C)	
				Max.	Min.
10/03/2018	1	j_2	No Penetration	30.0	13.0
11/03/2018	2	j_2	-do-	30.6	10.5
12/03/2018	3	j_2	Penetration started	31.2	11.0
15/03/2018	5	j_2 swollen	Swelling of roots started	32.6	14.5
20/03/2018	10	j_3	Galls formation	32.8	12.5
29/03/2018	19	j_4	-do-	35.0	12.5
03/04/2018	24	Female	-do-	36.3	23.0
06/04/2018	26	Male	-do-	35.6	19.5
10/04/2018	30	Eggs deposition	Egg masses	31.3	21.9
13/04/2018	33	j_2	-do-	33.9	14.0

Date of sowing: 03/03/2018; Date of inoculation: 09/03/2018

Life cycle of root-knot nematode, *M. incognita* on okra in the month of April-May

The observations presented in Table 2 revealed that under screen house conditions, j_2 penetration started at root tips on 2nd day. j_2 started swelling on 5thday (j_2 with spike tail) which became j_3 on 8th day and continued up to 16th day. On 17th day, j_4 stage was detected which started further swelling and after 5 days, female was observed on 22ndday. Males in the soil were observed on 24 DAI. Gravid female with egg masses were observed on 26th days. On 29 DAI, ten j_2 of the second generation were recorded inside the root. Thus, life cycle of the root-knot nematode, *M. incognita* from j_2-j_2 stage was completed in 39 days under screen house conditions in the month of April-May. Some of the second generation larvae (j_2) developed further inside the old galls while others invaded new roots and induced new galls at root tips. In spite of agricultural importance of root-knot nematodes few studies have focussed on the effects of temperature on the duration of life cycle. In present studies j_2 of *M. incognita* penetrated into young roots of okra plant near the root tip within 72 hours (h) in the month of March-April. Similarly, Mishra [4] also reported that *Heterodera zeae* j_2 penetrated roots within 3 hrs of inoculation, complete penetration within 6 hrs and move to the central portion of root within 5 days but temperature was not mention.

Table 2: Time taken for life cycle of root-knot nematode, *M. incognita* on okra in the month of April-May

Date	Observations (Days after inoculation)	Developmental stage of nematode	Remarks	Temperature (^{0}C)	
				Max.	Min.
11/04/2018	1	j_2	No Penetration	34.4	19.4
12/04/2018	2	j_2	Penetration started	28.4	17.5
13/04/2018	3	j_2	Swelling of roots started	33.9	14.0
15/04/2018	5	j_2 swollen	Galls formation	35.9	17.5
18/04/2018	8	j_3	-do-	38.0	20.0
27/04/2018	17	j_4	-do-	41.2	23.5
02/05/2018	22	Female	-do-	39.6	27.0
04/05/2018	24	Male	-do-	36.9	26.0

06/05/2018	26	Eggs deposition	Egg masses	40.2	26.2
09/05/2018	29	j_2	-do-	35.4	20.9

Date of sowing: 04/04/2018; Date of inoculation: 10/04/2018

Similar findings were also reported by Ustinov and Tereshchenko [11] in *Ditylenchus destructor* and observed that, temperature influenced the life cycle of *D. destructor*. Vrain [12] studied the effect of low temperature on the development of *M. incognita* and reported that the life cycle was completed under clover at 20 ^{0}C, but not at 16, 12, 8 ^{0}C. Contrary to it, second-stage larvae developed into adults 14 DAI at the high, 18 days at the intermediate (21.1 °C night and 26.7 °C day) and 34 days at the low temperature regime (15.5 °C night and 21.1 °C day). Eggs were observed 20 DAI at the high, 26 days at the intermediate and 54 days at the low temperature regime [13]. In South Africa, 56 days required to complete in life cycle of *M. javanica* at a mean temperature of 14 ºC, compared with only 21 days at 26 °C [3]. In case of wheat crop, life cycle of *M. graminicola* completed in 23 day during the month of February and March [9].

M. incognita (j_2) took 5 days to reached stele region followed by 31±1 °C and 23±1 °C (7 days) while, at 21±1 °C, it took 10 days. Multiplication of *M. incognita* was greatly affected by temperature. Maximum number of galls per root system (119.6), number of egg masses per root system (275.2), number of eggs per egg mass (343.6), final nematode population (1335.2) and reproduction factor of nematodes (2.67) was observed at 27±1 °C followed by 31±1 °C, 23±1 °C and 21±1 °C respectively [10]. The first molt of *M. naasi* in wheat seedlings was in 8 to 11 days at 22 to 26º C, and infective second stage larvae hatched in 15 to 17 days. Large numbers of larvae penetrated roots in 24 hours and were sedentary in feeding position in 2 or 3 days and sex differentiation was visible after 12 days. Eggs were deposited starting the 30th to 40th day [7].

Conclusion

The present study indicates that the life cycle, development and reproduction of *M. incognita* on okra were profoundly affected by temperature. For the completion of life cycle, a difference of time period of 4 days in okra could be attributed to differential rate of nematode development as the temperature was more or less same for experimentation. Although higher temperatures can affect the rate of nematode development and thus nematode life cycle, our results showed that temperature substantially decrease or increase the rate of development of *M. incognita* on okra.

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