International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 2387-2391

Received: 03-03-2018
Accepted: 04-04-2018

Priyanka Duggal
Department of Nematology, CCS HAU Hisar, Haryana, India

SR Goel
Department of Nematology, CCS HAU Hisar, Haryana, India

Sharmishtha Thakur
Department of Nematology MPUAT, Udaipur Rajasthan, India

Correspondence
Priyanka Duggal
Department of Nematology
CCS HAU Hisar, Haryana, India

Life cycle and Pathogenicity of *Meloidogyne incognita* on *Fragaria vesca* under Polyhouse and screen-house conditions in Haryana

Priyanka Duggal, SR Goel, Sharmishtha Thakur

Abstract
The root-knot nematode is a serious problem in the cultivation under commercial polyhouse. To access the damage potential of the nematode, life cycle and pathogenicity of root-knot nematode was studied on strawberry in polyhouse as well as under screen house conditions. Nematode didn't complete their life cycle neither under screen house conditions nor in polyhouse as only one disintegrated female was observed under polyhouse condition during last observation at 60th days. The pathogenicity of root-knot nematode at the different inoculum levels *viz*., 0, 10, 100, 1,000 and 10,000 J_2 of *Meloidogyne incognita* /pot was studied and compared. The pathogenic level of root-knot nematode in strawberry was recorded at and above 1,000 J_2 inoculum level under both the conditions where significant reduction in plant growth parameters was recorded.

Keywords: Polyhouse, screen house, root-knot nematode, strawberry, life cycle, pathogenicity and growth parameters.

1. Introduction
Protected cultivation may be defined as the "modification of natural environment to achieve optimum plant growth" (Singh, 2005) [13], in which Root-knot nematode (*Meloidogyne* spp.) is becoming a limiting factor in the economic production of various horticultural crops in some parts of the country. In Haryana state, this problem is increasing and assuming alarming proportions. Nagesh & Reddy, 1996 [11] reported 26 and 30% yield loss on carnation and Gerbera respectively in commercial polyhouse. Considering the importance of this nematode, the present study on some aspects of host-parasite relationships of this nematode was conducted in strawberry (*Fragaria vesca*) crop which is a native of temperate region. As in tropical and sub-tropical conditions, *Meloidogyne incognita* is a serious problem so we studied this nematode in strawberry to find out whether it infests the crop or not.

Material and Methods
Present study was carried out to determine the host parasites relationship of root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in Strawberry under protected cultivation in polyhouse of the Department of Vegetable Science and screen house of the Department of Nematology, CCS Haryana Agricultural University, Hisar.

Propagation of pure culture of *Meloidogyne incognita* for obtaining eggmasses and J_2
For identification of root-knot nematode *Meloidogyne incognita*, galled brinjal roots were collected from the naturally infested soil of field lab, Department of Nematology and brought to the laboratory. Eggmasses were separated in sodium hypochlorite solution (0.4-0.5 per cent free chlorine) after continuous stirration for five minutes for detachment of eggmasses from the roots and eggs were collected in on 500 mesh sieve after proper washing to remove the excess sodium hypochlorite. The contents of 500 mesh sieve were taken in a beaker and placed on Modified Baermann's funnel technique for 24 hours. After that freshly hatched juveniles were collected. 20-30 matured females were dissected from these roots under stereoscopic microscope by teasing the roots with the help of needle and forceps for preparation of perineal pattern for identification of species. The posterior portion of these females was cut and inner content was removed softly. The perineal pattern was mounted on glass slides in a drop of lectophenol and covered with a cover slip and sealed with nail polish. Several such slides were

prepared, each having about 5-6 perineal pattern. Then, these slides were observed under compound microscope under oil immersion lens according to the key given by Taylor *et al.* (1955) [14]. The species, thus identified was *M. incognita* (Kofoid and White) Chitwood, 1949. After identification, pure culture was propagated with the eggmasses collected from root-knot nematode infected roots. About 40-50 earthen pots were filled with steam sterilized soil and four weeks old brinjal seedlings were raised in that pots and inoculated with *Meloidogyne incognita* juveniles. After 65 days of inoculation, wilted plants showed heavy galls in the roots. Some plants were selected and brought to the laboratory and the same process was repeated for identification of M. incognita females through perineal patterns. Eggmasses and juveniles from these plants were used in inoculation for further experimentation during the course of present investigations. The culture was periodically sub-cultured for multiplication and purity. The desired number of eggmasses collected by sodium hypochlorite method were transferred to double folded tissue paper held on a moulded piece of aluminium wire net placed on petri plate at 28±2 ℃ temperature. Sufficient amount of water was added to keep the eggmasses just submerged. On the next day, water from these petri plates containing second stage juveniles was collected in beakers. The number of juveniles was counted per ml solution with three replications. These freshly hatched juveniles were used for inoculation for further experimentation. Nematode inoculation was done carefully by removing the soil around the roots of the plant to ensure direct and easy approaches of juveniles to the roots system. The larval suspension was bubbled continuously for 10-15 seconds for uniform quantity and poured in the vicinity of exposed roots system according to the desired population, required for the both experiment. For life cycle experiment, 1,000 J_2 per pot (6" diameter pot with 1 kg soil capacity) and for pathogenicity experiment different inoculum levels viz., 0, 10, 100, 1,000 and 10,000 J_2 per pot with three sets of replications for each crop and treatments.

Life cycle of root-knot nematode in strawberry under screen house and polyhouse conditions

The observations presented in Fig. 1 revealed that under screen house conditions, penetration of J_2 was observed on 4th day of inoculation which continued up to 14th day and no further development was observed till 14th day. J_2 started swelling on 14th day and J_3 were detected on 17th day and no further development was observed up to 26th day. On 29th day, J_4 stage was observed in the roots. No development from J_4 stage was observed even up to 60th day. Under polyhouse conditions, J_2 penetrated from 4th day up to 11th day. Swollen J_2 was observed on 11th day which became J_3 on 14th day and continued up to 23rd day. From 26th day to 60th day, only J_4 stage was observed, however, only one disintegrated female was also observed on 54th day. On 60th day, only J_4 stage was found.

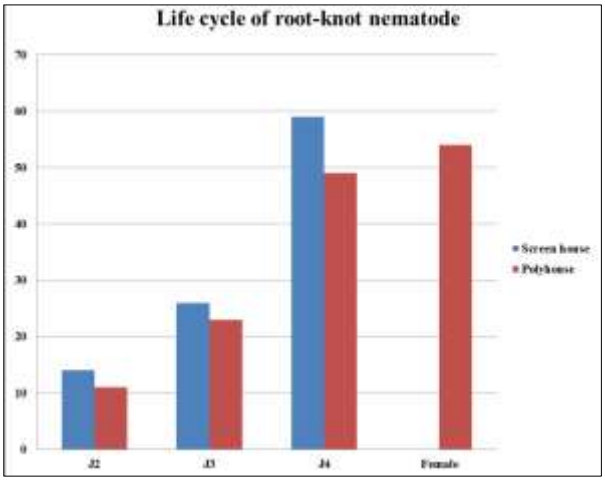

Fig 1: Comparison between life cycle of root-knot nematode under screen house and polyhouse conditions in strawberry

Pathogenicity of root-knot nematode in strawberry under screen house and Polyhouse conditions

The data recorded on various plant growth parameters and nematode reproduction factors of root-knot nematode, *Meloidogyne incognita* are presented in Table 1and 2 and Fig 2 and 3.

Under screen house conditions

The data in Table 1 revealed the effect of various levels of root-knot nematode on plant growth parameters nematode reproduction factors in strawberry under screen house conditions. The data in Table 4.7 revealed that maximum shoot length (16.0 cm) was observed in uninoculated control which differed significantly at 1,000 J_2 level (11.8 cm), being minimum (8.6 cm) at 10,000 J_2 level. Fresh shoot weight (15.6 g) was recorded in uninoculated control which was statistically at par up to 10 J_2 level. Minimum fresh shoot weight (9.3 g) was recorded at 10,000 J_2 level. But, the differences in fresh shoot weight at 1,000 (10.6 g) and 10,000 J_2 (9.3 g) inoculum levels were statistically non-significant with each other. Significant reduction in dry shoot weight was observed at and above 1,000 J_2 (2.7 g) as compared to uninoculated control (4.0 g), being minimum at 10,000 J_2 level (1.7 g), however, the differences at 1,000 (2.7 g) and 10,000 J_2 inoculum levels (1.7 g) were statistically non-significant with each other. Maximum dry shoot weight (4.0 g) was observed in control check. Fresh root weight was found maximum (13.7 g) in uninoculated control which differed significantly at and above 1,000 J_2 /plant (8.4 g), being minimum (6.8 g) at highest inoculum level of 10,000 J_2/plant. Maximum dry root weight (4.3 g) was recorded in uninoculated control. However, the differences at 1,000 (2.3 g) and 10,000 J_2 inoculum level (1.2 g) were statistically non-significant. Number of galls increased with increase in inoculum level, conspicuously at and above 1,000 J_2 level. Minimum number of galls (7.0/plant) was observed at

inoculum level of 1,000 J_2/plant, being maximum (16/plant) at highest inoculum level of 10,000 J_2/plant. No formation of eggmasses was observed in strawberry under screen house conditions up to 45th day so the final nematode population was not recorded.

Under polyhouse conditions

Data in Table 2 showed that maximum shoot length (17.3 cm). However, significant differences in shoot length was observed at and above 1,000 J_2 inoculum level (13.0 cm) which differed significantly from 10,000 J_2 inoculum level (10.3 cm). Fresh shoot weight was found maximum (18.8 g) at 0 inoculum level and significant differences in shoot weight were recorded at and above 100 J_2 level (15.1 g), being minimum at 10,000 J_2 inoculum level (11.2 g) followed by 1,000 J_2 inoculum level (13.7 g). Significant differences in dry shoot weight in uninoculated control (4.7 g/plant) were observed at and above inoculum level of 1,000 J_2/plant (3.0 g), being minimum (2.2 g) at inoculum level of 10,000 J_2/plant level Fresh root weight was found maximum (15.1 g) in uninoculated check, being minimum (9.0 g) at highest inoculum level of 10,000 J_2/plant and followed by 1,000 J_2 level (11.0 g). Significant reduction in dry root weight over uninoculated check (5.0 g) was observed at and above 1,000 J_2 level (2.5 g), being minimum (1.8 g) at 10,000 J_2 level/plant. Number of gall per root system was found maximum (32.3) at highest inoculum level of 10,000 J_2 followed by 1,000 J_2 level (19.7). No eggmass formation was recorded under polyhouse conditions also at any of the inoculum levels. No population of the root-knot nematode was recorded under polyhouse conditions at any of the inoculum level.

Table 1: Effect of root-knot nematode, *Meloidogyne incognita* on plant growth parameters and nematodes reproduction factor in strawberry under screen house conditions (Mean of three replicates)

Inoculum levels	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	No. of galls per root system	No. of eggmasses per root system	FNP (J_2 in soil)
0	16.0	15.6	4.0	13.7	4.3	0.00 (1.00)	-nil-	-nil-
10	15.3	14.9	3.7	11.3	3.3	0.00 (1.00)	-nil-	-nil-
100	14.0	12.2	3.0	10.1	2.5	0.00 (1.00)	-nil-	-nil-
1,000	11.8	10.6	2.7	8.4	2.3	7.00 (2.81)	-nil-	-nil-
10,000	8.6	9.3	1.7	6.8	1.2	16.00(4.10)	-nil-	-nil-
C.D.(P=0.05)	1.4	1.4	1.1	1.4	1.0	(0.4)	-	-

Figures in parentheses are √n+1 transformed value

Table 2: Effect of root-knot nematode, *Meloidogyne incognita* on plant growth parameters and nematodes reproduction factor in strawberry under polyhouse conditions (Mean of three replicates)

Inoculum levels	Shoot length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	No. of galls per root system	No. of eggmasses per root system	FNP (J_2 in soil)
0	17.3	18.8	4.7	15.1	5.0	0.0 (1.0)	-nil-	-nil-
10	16.9	17.6	4.3	13.8	4.3	0.0 (1.0)	-nil-	-nil-
100	15.0	15.1	3.7	12.6	3.7	7.0 (2.8)	-nil-	-nil-
1,000	13.0	13.7	3.0	11.0	2.5	19.7 (4.5)	-nil-	-nil-
10,000	10.3	11.2	2.2	9.0	1.8	32.3 (5.7)	-nil-	-nil-
C.D. (P=0.05)	2.0	2.1	1.0	2.1	0.5	(0.2)	-	-

Figures in parentheses are √n+1 transformed value

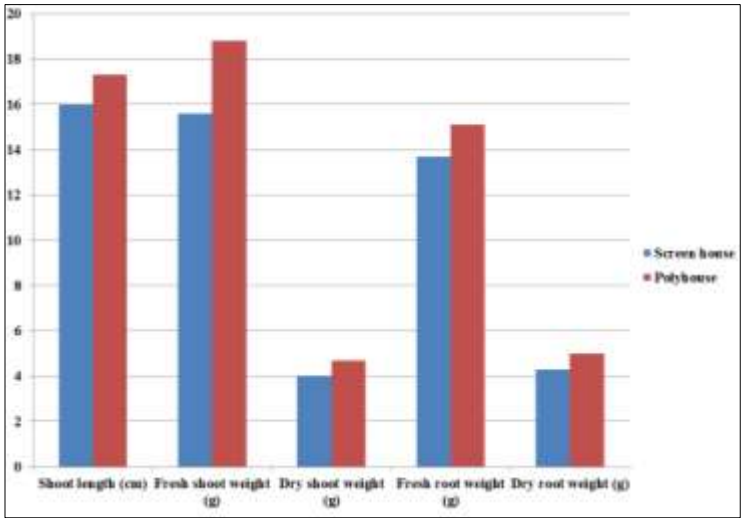

Fig 2: Comparison between growth parameters of strawberry under screen house and polyhouse conditions

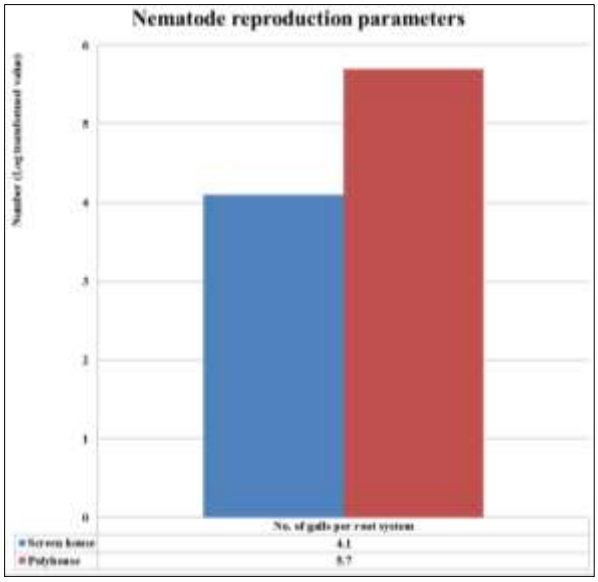

Fig 3: Comparison between root-knot nematode reproduction parameters under screen house and polyhouse conditions in strawberry

Discussion

Root-knot nematode (*Meloidogyne* spp.) is becoming a limiting factor in the economic production of various horticultural crops grown under protected conditions. On strawberry, when transplanted in November 2015, nematode did not penetrate even up to 60 day of inoculation in polyhouse as well as screen house conditions. However, nematode penetrated and developed further when transplanted again in March 2015 nematode. Nevertheless it was unable to complete its life cycle and only J_4 stage was recorded up to 60 days of inoculation in screen house conditions. In polyhouse, nematode also penetrated and only one female was recorded on 54th day of inoculation. However, majority of nematodes were disintegrated at J_4 stage in decaying galls in both the experimental conditions. The differences in the development at two dates of sowing may be correlated with temperature 1.1-28.6 °C in November sown crop and 5.7-33.7 °C temperature in March sown crop. Strawberry being a temperate crop and recommended for sowing in generally in mid-September to mid-October under Haryana conditions. In the present study the crop was grown in mid-November in one set of experiment. Nematode failed to penetrate. These results may be exploited to manage root-knot nematode problem in strawberry in Haryana. Nematode penetrated and developed up to J_4 stages, when the crop sown in mid-March. Temperature (10.3-40 °C) prevailing at this time is not suitable for strawberry crop and roots of the plants started decaying. The unfavourable temperature for crop also adversely affected nematode development and reproduction. The results of strawberry crops are somewhat in agreement with those of Kanwar *et al*. (2008) [8] they studied the life cycle of *Meloidogyne graminicola* on wheat at two sowing times under greenhouse conditions in Hisar in India. In October-sown plants, J_2 readily penetrated roots, developed to adult female in 24 days and started laying eggs in 38 days. However, majority of egg hatched only in the month of February. Comparatively fewer J_2 penetrated in November sown plants and developed slowly to adulthood in 100 days. Egg and J_2 of next generation was recorded 110 days after inoculation. The pathogenic level in strawberry was observed at and above 1,000 J_2 inoculum level but maximum growth parameters were recorded under polyhouse conditions as compared to screen house conditions. No eggmass formation was recorded in strawberry under either of the conditions. The results of pathogenicity of root-knot nematode at the prescribed inoculum levels are in agreement with that of Ansari *et al*. (2012) [1] who conducted an experiments under net house condition to determine the individual effect of different inoculum levels of root-knot nematode, *Meloidogyne incognita*, Race-2 on plant growth parameters viz., Plant length, fresh and dry weights and number of fruits of tomato var. P21. The threshold level of root-knot nematode was 1000 J_2/kg soil. Inoculum level of *Meloidogyne incognita* race-2 was pathogenic at and above 1000 J_2/kg soil and caused significant reduction. Choi *et al*. (1992) [2] who studied the effects of *Pratylenchus coffeae* on growth of *Gerbera jamesonii* cv. Teracombi in a greenhouse. At inoculum levels of 1,000, 5,000 and 10,000 nematodes, fresh shoot and root weights were reduced by 69, 76 and 85 per cent. Six months after planting, plants inoculated with 1000 and 5000 nematodes, the number of flowers decreased by 81 per cent and 95 per cent respectively, while 10,000 nematodes per plant produced no flowers. Dhankar *et al*. (1986) [3] who reported that the threshold inoculum levels of *M. incognita* on water melon (*Citrullus vulgaris* Scharad) was 1000 larvae/kg soil. At this inoculum levels all the plant growth parameters were significantly reduced over control. Ganaie *et al*. (2011) [4] who observed a significant reduction in various plant growth parameters of okra at and above the inoculum level of 1000 J_2 / 2 kg soil of *M. incognita*. Gupta *et al*. (1995) [5] who reported the effect of various initial inoculum levels viz., 0, 10, 100, 1000, 2000, 5000 and 10, 000 J_2 of *Meloidogyne spp*. /kg soil on some cucurbitaceous crops. They found significant reduction in growth of all the crops at initial inoculum level of 1000 J_2/pot. Galling was found maximum at highest initial population density. Hazarika & Phukhan (1994) [6] also reported that the significant reduction in height, root and shoot weight of plants was at an inoculum level of 1000 nematodes/plant.

Johnson *et al*. (2003) [7] reported the damage potential and pathogenic levels of *M. incognita* on gladiolus and carnation under glasshouse conditions. Growth parameters like shoot and root length, shoot and root weight and number of leaves were significantly reduced by different inoculum levels (10, 100 and 10000 J_2/plant) of *M. incognita* in both gladiolus and carnation. The reproduction rate of *M. incognita* on these crops was drastically reduced at higher inoculum levels (10,000 J_2/plant). It was also observed that even 100 J_2/plant

were able to cause economic damage to gladiolus and carnation. Khanna & Jyoti (2004) [9] reported the pathogenic potential of *M. incognita* on *Dianthus caryophillus*. Plant growth was significantly reduced at the levels of 1,000 and 10,000 juveniles as compared to control. Meena & Mishra (1993) [10] observed *M. incognita* causes a significant reduction in almost all the plant growth parameters with increase in the levels of nematode inoculum. Maximum reduction in all the plant growth parameters was recorded at the inoculum level of 10,000 J_2/pot which was followed by the levels of 1000 J_2/pot.

Refrences

1. Ansari S, Shahab S, Mazid M, Ahmed D. Comparative study of *Fusarium oxysporum* f sp. *lycopersici* and *Meloidogyne incognita* race-2 on plant growth parameters of tomato. Agricultural Sciences. 2012; 3: 844-847.
2. Choi D, Choi YM, Han SC. Effects of Pratylenchus coffeae on growth of gerbera. Research Reports of the Rural Development Administration. Crop Protection Agricultural Science Institute. 1992; 34:85-90.
3. Dhankhar RKS, Sharma C, Sarna NT, Trivedi PC. Pathogenicity of *Meloidogyne* incognita on water melon (Citrullus vulgaris Scharad). Indian Journal of Nematology. 1986; 16:274.
4. Ganaie MA, Khan TA. Studies on the interactive effect of *Meloidogyne incognita* and *Fusarium solani* on *Lycopersicon esculentum*, Mill. International Journal of Botany. 2011; 7:205-208.
5. Gupta DC, Paruthi IJ, Jain RK. Effect of initial inoculum levels of *Meloidogyne* spp. on som cucurbitaceous crops. Indian Journal of Nematology. 1995; 25:194-199.
6. Hazarika K, Phukhan PN. Studies on the pathogenicity of *Meloidogyne incognita* on brinjal. Journal of Agricultural Sciences. 1994; 7; 221-223.
7. Johnson SBN, Cannayane I, Rajendran G. Studies on pathogenic level of *Meloidogyne incognita* on Gladiolus and Carnation. Current Nematology. 2003; 14:75-78.
8. Kanwar RS, Dabur KR, Bajaj HK, Nandal SN. Life cycle of *Meloidogyne graminicola* and its possibility of becoming a pest of wheat crop. Pakistan Journal of Nematology. 2008; 26:45-50.
9. Khanna AS, Jyoti J. Pathogenicity of *Meloidogyne incognita* on Dianthus caryophillus. Nematology Mediterranea. 2004; 32(1):125-126.
10. Meena ML, Mishra SD. Pathogenicity potentiality and extent of damage to soybean by root-knot nematode, *Meloidogyne* incognita. Current Nematology. 1993; 4: 1-6.
11. Nagesh M, Parvatha Reddy P. Management of rootknot nematodes on carnation using antagonistic fungi and neem cake. National Seminar on Integrated crop Disease Management on Lycopersicon esculentum, Mill. International Journal of Botany. 1996; 7: 205-208.
12. Singh B. Protected cultivation of vegetable crops. Kalyani Publisher, 2005, 168.
13. Taylor AL, VH, Dropkin, GC Martin, Perineal patterns of root-knot nematodes. Phytopathology. 1955; 45:26–34.