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Pathogenicity studies on root-knot nematode, meloidogyne incognita race-II chitwood, infesting pomegranate in glasshouse

Kadam DB, Band SS and Palande PR

Abstract
In Pathogenicity studies on root-knot nematode, Meloidogyne incognita race-II Chitwood, infesting Pomegranate in glasshouse, two months old rooted grafts of pomegranate (cv. Bhagwa) were inoculated with 0, 10, 50, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10,000 nematodes per plant containing 2nd stage juveniles of root- knot nematodes. The experiment was terminated 45 days after inoculation. It was observed from the results that with increase in the inoculation level of root-knot nematode, there was progressive decrease in plant growth with significant reduction in all the plant growth characters and increase in nematode multiplication factor at higher inoculation levels. The significant reduction in length, fresh and dry weights of shoot and root and significant increase in nematode multiplication factors was observed at 1000 and above inoculation levels. However, the number of times nematode multiplication decreased with increase in inoculum level.

Keywords: Meloidogyne incognita, pathogenicity, nematodes, length, fresh weight, dry weight etc.

Introduction
Pomegranate important fruit crop is attacked by several insect and non-insect pests as well as diseases. Diseases caused by nematodes are of economic importance. Darekar *et al*. (1990) [3] reported 10 species of plant parasitic nematodes associated with this crop in Maharashtra state. The root-knot nematode, Meloidogyne incognita Chitwood, 1949 [2] is one of them causing considerable yield loss in pomegranate. The root-knot nematodes, Meloidogyne spp. are basically parasites of roots causes root galls or knots, as a below ground symptoms. The above ground symptoms, are those of slow debility of roots in its function of nutrient and water uptake and translocation. The plants may be stunted, yellowish with smaller foliage and poor and fewer fruits. The symptoms are often mistaken for macro or micro- nutrient deficiency or moisture stress. Besides, the direct damage caused to the plant, the root-knot nematodes are notorious for the disease complexes involving fungi, bacteria, virus, mycoplasma, insects and other nematodes (Dasgupta and Gaur, 1986) [4]. In general, the root-knot nematode, M. incognita is detrimental to pomegranate by depressing the plant growth and thereby reducing the yield. So far no systemic efforts have been made to manage this melody. Therefore, there is an urgent need to undertake the research on this important nematode infesting pomegranate with above aspects.

Material and Methods
Pathogenicity studies on root-knot nematode, Meloidogyne incognita race-II Chitwood, infesting Pomegranate in glasshouse, a statistically designed experiment was in the glasshouse of AICRP on Nematodes, Department of Agricultural Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri. Seedlings of pomegranate cv. Bhagwa were planted in 15 cm diameter earthen pots containing a mixture of 1 kg autoclaved soil and FYM in 3:1 ratio. Ten days after planting, inoculation was carried out by keeping only one healthy seedling per pot. For inoculation, the stock culture of the nematode was maintained on tomato in micro plots. Inoculation was done by pouring the freshly hatched nematode suspension obtained from egg masses of a stock culture in a three holes prepared around the plant and on roots exposed by removing the top layer of the soil, which were later on covered by the moist autoclaved soil. Before inoculation the nematode count per ml of suspension of the culture was taken under steriozoom binocular microscope and required quantity of the suspension poured into the pots according to inoculums levels.

The plants were watered periodically and were given the recommended dose of fertilizers. The experiment was conducted in Completely Randomized Block Design with three replications and fifteen treatments. The details of the treatments are given below.

Sr. No.	:	Treatment
1	:	0 nematodes (J2) /plant/pot
2	:	10 nematodes (J2)/plant/pot
3	:	50 nematodes (J2)/plant/pot
4	:	100 nematodes (J2)/plant/pot
5	:	500 nematodes (J2)/plant/pot
6	:	1000 nematodes (J2)/plant/pot
7	:	2000 nematodes (J2)/plant/pot
8	:	3000 nematodes (J2)/plant/pot
9	:	4000 nematodes (J2)/plant/pot
10	:	5000 nematodes (J2)/plant/pot
11	:	6000 nematodes (J2)/plant/pot
12	:	7000 nematodes (J2)/plant/pot
13	:	8000 nematodes (J2)/plant/pot
14	:	9000 nematodes (J2)/plant/pot
15	:	10000 nematodes (J2)/plant/pot

Method of recording observations

The experiment was terminated 60 days after the inoculation. The plants were uprooted and roots were washed with clean tap water to make free from the soil particles. The plants were then cut at the base observations like shoot and root lengths, shoot and root fresh and dry weights and number of galls per plant were recorded. The observations on shoot and root lengths were recorded to complete full figures of cm by adjusting the decimals, while, the fresh and dry weights were recorded upto two decimals of gram on top pan balance. The number of galls with and without egg masses were recorded after staining the roots in 1.0 percent solution of trypan blue for two minutes (Franklin, 1949). The observations on total number of females were recorded on one gram of roots for convenience and to maintain the accuracy. Thus, the figures obtained were multiplied by the corresponding fresh weight of roots to have the number of galls per plant. Three well developed egg masses from each inoculated treatments were selected and squeezed in lactophenol slowly with a needle under the binocular microscope and number of eggs per egg mass were counted and recorded by averaging the total of three egg masses and with proper multiplication factor the total number of eggs per plant were calculated. The soil samples from the pots were washed in the laboratory with Cobb's Sieving and Decanting Method and counts of final root-knot nematode populations were recorded. For this purpose, the residues of 200 and 350 mesh sieves were collected in plastic beaker and the volume of beaker was adjusted to 200 ml by adding tap water. For nematode count, the average of 10 counts of 1 ml suspension was taken and from this it was calculated to 200 ml of suspension which was the soil population in the pot. Thus, total nematode population and the reproduction rate (Pf/Pi) were calculated. For dry weights, the shoot and roots of the plants of different inoculum levels were kept in hot air oven at 60 0C till the constant weights were obtained.

Analysis of the experimental data

In order to find out significant differences in the different inoculums levels, all the experimental data were statistically analyzed. The significance of different inoculation levels was assessed at 5 percent level.

Results and Discussion

Pathogenicity of root-knot nematode, M. incognita race-II was studied on pomegranate cv. Bhagwa on which the effect of different initial inoculums levels *viz*., 10, 50, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10000 nematodes per plant per pot containing 1 kg autoclaved soil was compared with uninoculated control for plant growth and nematode multiplication.

Plant growth characters

The observations on length, fresh and dry weight of shoot and root as a plant growth characters and number of root galls, egg masses, number of eggs/egg mass, total eggs/plant and nematode population in soil as nematode multiplication factors were recorded. The data obtained are presented in table 1 to 5.

It could be seen from the data that with the increased level of inoculation, there was a progressive decrease in plant growth characters. However, there were no significant differences in shoot, root and total plant length at 10, 50, 100 and 500 inoculums levels. The reduction in shoot, root and total plant length recognized in these inoculums levels ranged from 3.82 to 12.22; 6.21 to 13.27 and 4.93 to 12.71 percent, respectively. The significant reduction in shoot, root and total plant length was observed at 1000 and above inoculums level. The reduction in shoot, root and total plant length at 1000 inoculums levels was 17.56, 23.02 and 20.19 percent, respectively. The maximum reduction of 42.00, 48.69 and 45.09 percent in shoot, root and total plant length, respectively was observed at 10,000 inoculums level. In the case of fresh weight of shoot, root and total plant, it is evident from the Table 2 indicated that there was reduction in the fresh weight with the increase in the inoculums level. However, the significant differences were not observed in fresh weight of shoot, root and total plant at 10, 50, 100 and 500 inoculums levels. At these inoculums levels, the reduction in shoot, root and total plant fresh weight were 2.00, 3.67 and 2.40, 5.23, 8.01 and 5.87, 7.49, 8.21 and 7.97 and 10.10, 10.45 and 10.19 percent, respectively. The significant reduction in fresh weight of shoot, root and total plant was observed at 1000 and above inoculums levels. At 1000 inoculums level the reduction of 24.49, 28.31 and 25.38 percent in fresh weight of shoot, root and total plant, respectively was observed.

It could be seen from the table 3 that dry weight of shoot, root and total plant of pomegranate was reduced with the increase in the inoculums level. However, there were no significant differences in dry weight of shoot, root and total plant at 10, 50, 100 and 500 inoculums levels. The reduction in shoot, root and total plant dry weight recorded in these inoculums levels ranged from 4.74 to 14.57, 3.94 to 11.76 and 4.60 to 13.93 percent, respectively. The significant reduction in dry weight of shoot, root and total plant was observed at 1000 and above inoculums levels. The maximum reduction of 62.84, 64.71 and 64.60 percent in shoot, root and total plant dry weight respectively was recorded at 10,000 inoculums level.

Table 1: Effect of different inoculums levels of root-knot nematode, M. incognita race-II on plant growth characters of pomegranate

Sr. No.	Inoculum levels (No. of nematodes (J2)/ plant/ pot	Shoot length (cm/plant)*	Root length (cm/plant)*	Plant length (cm)*
1	0	43.67	37.67	81.34
		(0.00)	(0.00)	(0.00)
2.	10	42.00	35.33	77.33
		(3.82)	(6.21)	(4.93)
3.	50	41.33	34.33	75.66
		(5.36)	(8.87)	(6.98)
4.	100	40.67	33.67	74.34
		(6.87)	(10.67)	(8.60)
5.	500	38.33	32.67	71.00
		(12.22)	(13.27)	(12.71)
6.	1000	36.00	29.00	65.00
		(17.56)	(23.02)	(20.09)
7.	2000	34.67	28.67	63.34
		(20.61)	(23.89)	(22.13)
8.	3000	33.33	27.67	61.00
		(23.68)	(26.55)	(25.01)
9.	4000	33.00	27.00	60
		(24.43)	(28.33)	(26.24)
10.	5000	31.33	26.33	57.66
		(28.26)	(30.10)	(29.11)
11.	6000	30.33	25.33	55.66
		(30.54)	(32.76)	(31.57)
12.	7000	29.67	24.67	54.34
		(32.06)	(34.51)	(33.19)
13.	8000	26.33	23.00	51.33
		(39.70)	(38.94)	(30.01)
14.	9000	26.17	20.67	46.84
		(40.07)	(45.13)	(42.41)
15.	10000	25.33	19.33	44.66
		(42.00)	(48.69)	(45.09)
	S.E. +	2.02	1.62	3.42
	CD at 5%	6.54	4.68	5.66
	CV %	8.14	7.01	7.11

* Figures in parentheses are percent reduction over an untreated control

Table 2: Effect of different inoculum levels of root-knot nematode, M. incognita race-II on plant growth characters of pomegranate

Sr. No.	Inoculum levels (No. of nematodes (J2)/ plant/ pot	Shoot fresh weight (g/plant)*	Root fresh weight (g/plant)*	Plant fresh weight (g)*
1	0	48.99	14.73	63.72
		(0.00)	(0.00)	(0.00)
2.	10	48.00	14.19	62.19
		(2.00)	(3.67)	(2.40)
3.	50	46.43	13.55	59.98
		(5.23)	(8.01)	(5.87)
4.	100	45.32	13.32	58.64
		(7.49)	(8.21)	(7.97)
5.	500	44.04	13.19	57.23
		(10.10)	(10.45)	(10.19)
6.	1000	36.99	10.56	47.55
		(24.49)	(28.31)	(25.38)
7.	2000	34.75	9.66	44.41
		(29.07)	(34.42)	(30.30)
8.	3000	31.64	8.72	40.36
		(34.42)	(40.80)	(36.67)
9.	4000	29.36	8.33	37.69
		(40.07)	(43.45)	(40.85)
10.	5000	27.53	7.59	35.12
		(43.80)	(48.47)	(44.88)
11.	6000	26.53	6.96	33.49
		(45.85)	(52.75)	(47.44)
12.	7000	24.48	6.49	30.97
		(50.03)	(55.94)	(51.40)
13.	8000	23.52	5.99	29.51

		(51.99)	(59.33)	(53.69)
14.	9000	21.18	5.66	26.84
		(55.50)	(61.58)	(57.88)
15.	10000	19.96	5.39	25.35
		(59.26)	(63.40)	(60.22)
	S.E. +	0.48	0.16	0.61
	CD at 5%	1.37	0.46	1.77
	CV %	7.24	7.53	6.13

*Figures in parentheses are percent reduction over an untreated control

Table 3: Effect of different inoculum levels of root-knot nematode, M. incognita race-II on plant growth characters of pomegranate

Sr. No.	Inoculum levels (No. of nematodes (J2)/ plant/ pot	Shoot dry weight (g/plant)*	Root dry weight (g/plant)*	Plant dry weight (g)*
1	0	11.60	3.40	15.00
		(0.00)	(0.00)	(0.00)
2.	10	11.05	3.26	14.31
		(4.74)	(3.94)	(4.60)
3.	50	10.72	3.20	13.92
		(7.59)	(5.88)	(7.20)
4.	100	10.09	3.06	13.15
		(13.02)	(10.00)	(12.33)
5.	500	9.91	3.00	12.91
		(14.57)	(11.76)	(13.93)
6.	1000	8.04	2.43	10.47
		(30.70)	(28.53)	(30.20)
7.	2000	7.34	2.26	9.60
		(36.72)	(33.53)	(36.00)
8.	3000	7.05	2.16	9.21
		(39.22)	(36.47)	(38.60)
9.	4000	6.68	2.03	8.71
		(42.41)	(40.29)	(41.93)
10.	5000	6.20	1.93	8.13
		(46.55)	(43.24)	(45.80)
11.	6000	5.87	1.73	7.60
		(49.40)	(49.12)	(49.33)
12.	7000	5.51	1.53	7.04
		(52.50)	(55.00)	(53.07)
13.	8000	4.99	1.40	6.39
		(56.98)	(58.82)	(57.40)
14.	9000	4.56	1.30	5.86
		(60.69)	(61.76)	(60.93)
15.	10000	4.11	1.20	5.31
		(62.84)	(64.71)	(64.60)
	S.E. +	0.17	0.06	0.21
	CD at 5%	0.48	0.16	0.59
	CV %	8.11	8.13	7.33

*Figures in parentheses are percent reduction over an untreated control

Factors of reproduction

The nematode population (number of galls with and without egg masses, eggs per plant and soil population per pot) when estimated after 45 days of inoculation increased with an increase in inoculums level. The significant increase in number of galls was observed among all the levels of inoculums in comparison to uninoculated control (table 4). However, the inoculums levels of 500, 1000 and 2000 were on par with each other in recording galls population of 104.68, 147.68 and 271.34 per plant, respectively. The maximum numbers of galls of 903.33 per plant were recorded in the inoculums level of 10000, which was at par to the inoculums levels of 3000 to 9000. The number of galls/ plant recorded in these inoculums levels ranged from 397.34 to 819.68 per plant.

The average number of egg masses formed on roots with different inoculums levels varied from 1.44 to 175.23 per plant per pot. The number increased with the increase in inoculums from 10 to 10000. However, the inoculums level of 10000 recorded the maximum 175.23 egg masses per plant when compared to uninoculated control and rest of the inoculums levels. It could be seen from the table 4 that the differences in number of eggs per egg mass with different levels of inoculums were significant over uninoculated control. The number of eggs per egg mass with different inoculums levels varied from

421.00 to 497.92, the maximum (497.92) being at 10 inoculums level and the minimum (421.00) at 10,000 inoculums level. The number of eggs per egg mass in rest of the inoculums levels ranged from 428 to 49.

It is observed from the table 4 that with the increase in inoculums level there was progressive increase in the number of eggs per plant. The total number of eggs per plant ranged from 717.00 to 73772.00 with 10 to 10000 inoculums levels,

respectively. The maximum number of eggs of 73772.00 per plant was recorded in the inoculums level of 10,000 which was on par with the inoculums levels from 4000 to 9000. The number of eggs per plant recorded in these inoculums levels ranged from 60331.00 to 73350.00.In general, the progressive increase in root population of nematode was observed with the increase in the inoculums level. However, significantly minimum root population of nematode of 717.00 per plant was recorded in the inoculums level of 10. The maximum root population of nematode of 74675.33 per plant was observed in the inoculums level of 10000, which was on par with the inoculums levels of 5000 to 9000. The root population of nematode recorded in these inoculums levels ranged from 68304.00 to 74169.68 (table 5).

It is revealed from the table 5 that the soil population of nematode at different inoculums levels increased with the increase in inoculums level and ranged from 260.67 to 14272.67 from 10 to 10000 inoculums level. The initial inoculums levels from 10 to 10000 significantly differed from each other in recording soil population of nematode. The inoculums levels of 9000 and 10000 were significantly on par with each other in recording maximum soil population of 13561.67 to 14272.67 nematodes per pot.

Table 4: Effect of different inoculum levels of root-knot nematodes, M. incognita race- II on nematode multiplication factors of pomegranate

Sr. No.	Inoculum levels (No. of nematodes (J2)/plant/pot	Number of galls/ plant	Number of egg masses /plant	Number of egg/ egg masses	Number of eggs/plant
1	0	0	0	0	0
2.	10	5.30 (0.724)	1.44 (0.158)	497.92 (2.697)	717.00 (2.855)
3.	50	15.45 (1.188)	4.40 (0.643)	496.00 (2.695)	2182.40 (3.338)
4.	100	29.45 (1.4169)	8.42 (0.925)	490.00 (2.690)	4125.80 (3.615)
5.	500	104.68 (2.019)	31.68 (1.500)	484.00 (2.684)	15333.12 (4.185)
6.	1000	147.68 (2.169)	42.13 (1.624)	477.00 (2.678)	20096.01 (4.303)
7.	2000	271.34 (2.433)	73.32 (1.865)	471.00 (2.673)	34533.72 (4.538)
8.	3000	397.34 (2.599)	108.68 (2.036)	465.00 (2.667)	50536.00 (4.703)
9.	4000	522.34 (2.717)	130.87 (2.116)	461.00 (2.663)	60331.00 (4.780)
10.	5000	602.00 (2.779)	148.47 (2.171)	456.00 (2.658)	67702.00 (4.8300
11.	6000	683.68 (2.834)	181.32 (2.258)	451.00 (2.654)	72755.00 (4.862)
12.	7000	698.00 (2.843)	164.78 (2.216)	445.00 (2.648)	73327.00 (4.865)
13.	8000	752.00 (2.876)	165.88 (2.219)	434.00 (2.637)	71991.00 (4.857)
14.	9000	819.68 (2.913)	171.38 (2.233)	428.00 (2.631)	73350.00 (4.865)
15.	10000	903.33 (2.955)	175.23 (2.243)	421.00 (2.631)	73772.00 (4.837)
	S.E. +	0.156	0.019	0.023	0.090
	CD at 5%	0.450	0.056	0.067	0.259
	CV %	7.265	2.038	1.309	2.50

*Figures in parentheses are log (x + 1) transformed values.

Table 5: Effect of different inoculum levels of root-knot nematodes, M. incognita race- II on nematode multiplication factors of pomegranate

Sr. No.	Inoculum levels (No. of nematodes (J2)/ plant/ pot	Root population (No. of galls and eggs/ plant)	Soil population/ plant/pot	Root and soil population/ plant/pot	Rate of multiplication (RF/P)
1	0	- 0	- 0	- 0	- 0
2.	10	717.00 (2.855)	260.67 (2.416)	977.67 (2.990)	97.77
3.	50	2197.85 (3.342)	1370.00 (3.136)	3567.85 (3.552)	71.36
4.	100	4155.25 (3.618)	2686.00 (3.429)	6841.25 (3.835)	68.41
5.	500	15437.80 (4.188)	4871.81 (3.687)	20309.61 (4.307)	30.88
6.	1000	20243.69 (4.306)	8010.60 (3.903)	28254.29 (4.451)	28.25

7.	2000	34805.06	8194.93	42999.99	21.50
		(4.541)	(3.913)	(4.705)	
8.	3000	50733.34	8674.20	59407.54	19.80
		(3.938)	(3.938)	(4.773)	
9.	4000	60853.34	9169.27	70022.61	17.50
		(4.784)	(3.962)	(4.845)	
10.	5000	68304.00	9616.93	77920.93	15.57
		(4.834)	(3.983)	(4.891)	
11.	6000	73438.60	10322.67	84472.35	14.08
		(4.866)	(4.013)	(4.927)	
12.	7000	74025.00	11033.67	83761.35	11.97
		(4.869)	(4.043)	(4.923)	
13.	8000	72743.00	12245.00	84988.00	10.62
		(4.862)	(4.088)	(4.929)	
14.	9000	74169.68	13561.67	87731.35	9.75
		(4.870)	(4.132)	(4.943)	
15.	10000	74675.33	14272.67	88948.00	8.89
		(4.873)	(4.154)	(4.949)	
	S.E. +	0.027	0.021	0.014	
	CD at 5%	0.79	0.060	0.041	
	CV %	1.76	0.577	0.360	

*Figures in parentheses are log (x + 1) transformed values.

In case of total nematode population and its number of times multiplication, it is evident from table 5 that there was a significant increase in the total nematode population with an increase in the inoculum levels but the number of times multiplication of the nematode was decreased with an increase in the inoculum levels. The nematode count ranged from 977.67 to 88948.00 per plant per pot from the inoculum levels of 10 to 10000, respectively. However, the number of times nematode multiplication ranged from 97.77 to 8.89 from the inoculum level of 10 to 10000, respectively. This may be due to fact that there was competition for food among the nematodes. It was further observed that at 10, 50 and 100 inoculum levels, the multiplication rate of the nematode was greater i.e. 97.77, 71.36 and 68.41, respectively than the 500 and above inoculum levels. In general, it was observed that the inoculum level of 10, 50, 100 and 500 nematodes per plant had very little effect on the plant growth characters *viz*., shoot and root length, fresh and dry weights and nematode multiplication factors *viz*., number of galls/females, egg masses, eggs and soil population of nematodes. The inoculum level of 1000 and above nematodes significantly affected the plant growth and nematode multiplication factors.

Before, deciding any Micro Organism to be a pathogen, it is necessary to prove its pathogenicity with the host to which it is to be said as pathogen. For every pathogen, there is a level called economic injury level beyond which one cannot tolerate losses caused by a pathogen and need to control it by any means. The behaviour of plant parasitic nematodes varies with their habits of parasitism. The nematodes with full or partial habits of endoparasitism are more dangerous even at few numbers. The root-knot nematode is one of such group of nematodes. Pomegranate one of the susceptible hosts of the root- knot nematode is important fruit crop gaining more and more importance in present days and so it was selected for such studies to have information which could help in applying suitable control measures. The experiment with nematode inoculums levels 10, 50, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000 and with control (uninoculated) as check was conducted on a pomegranate cv. Bhagwa to find out the number of nematodes per plant per pot containing 1 kg autoclaved soil, which caused significant damage to the crop. Results indicated that the level of 1000 or more nematodes per plant significantly damaged the plant. The plant growth characters measuring pathogenicity *viz*; shoot and root length, fresh and dry weights observed to be reduced significantly at this level of inoculums. Similar reduction in shoot and root length and weight of pea cv. Boneville was reported by Bhagawati and Phukan (1991) [1] in mulberry (*Morus alba* L.) by Govindaih *et al*. (1991) [5] in sunflower, safflower and mustard by Prasad and Chawal (1992) in potato by Kantharaju and Reddy (2001) [6].

Conclusion

Thus, from the experiments on pathogenicity it can be very well concluded that a plant of pomegranate damaged by the root-knot nematode, M. incognita race-II with the inoculums of 1000 nematodes per pot containing 1 kg autoclaved soil, i.e. 1 nematode per g of soils.

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