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Studies on incidence of root knot nematode and its bio management in pomegranate (*Punica granatum* L.)

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ABSTRACT: The community analysis of nematodes associated with pomegranate inferred that the relative frequency, relative density and prominence value were the highest for root knot nematode, *Meloidogyne incognita* followed by reniform nematode, *Rotylenchulus reniformis*. Pathogenicity studies revealed that 10,000 juveniles of root knot nematode caused maximum reduction in growth parameters with highest gall index. Histopathological studies of root knot nematode, *M.incognita* infested root sections showed root knot nematode, *M.incognita* females lodged at the pericylce region forming giant cells with denser cytoplasm and multinucleate condition. Field studies conducted at Theethipalayam in pomegranate var. Bahuwa infested with root knot nematode, *M.incognita* with more than one juvenile per gram of soil revealed that application of bioagent, *Purpureocillium lilacinum*@ 15 g/plant (cfu 2 x 10^6) applied thrice every three months' interval along with FYM was effective in reducing population of root knot nematode, and in increasing yield considerably. However, it was on par with *Bacillus subtilis* at the same dose.

Keywords: Community analysis, root knot nematode, pathogenicity, histopathology, management, bioagent.

INTRODUCTION

Of late, fruit crops are becoming more prone to plant parasitic nematodes especially root knot nematode, due to intensification of horticulture. While nurseries are playing a pivotal role in transmitting nematodes through infested planting materials, awareness is being created amongst the nursery men on the presence of nematodes in their material and ways to prevent them through trainings and workshops in the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore. Recently, four new records of root knot nematode belonging to various species have been published by the Department of Nematology, TNAU, Coimbatore that includes *Meloidgyne enterolobii* from guava (Poornima *et al*., 2016), *M.incognita* from pomegranate (Illangovan and Poornima, 2016), *M. indica* from citrus and *M.incognita* and *M.arenaria* from mango (Poornima *et al*., 2017). Sasser and Freckman (1979) have reported that among the plant parasitic nematodes, root knot nematode (*Meloidogyne* spp.) is found to be worldwide in distribution and affects many economically important crops. Many species of plant parasitic nematodes are associated with pomegranate in India (Chadha and Pareek, 1993) and some of the species are highly damaging to pomegranate production (Darekar *et al.*, 1990). Nematodes pave way for entry of disease causing microorganisms causing disease complex in crops. Atkinson's, 1982 recorded that root knot nematodes always increase the severity of *Fuasrium* wilt.

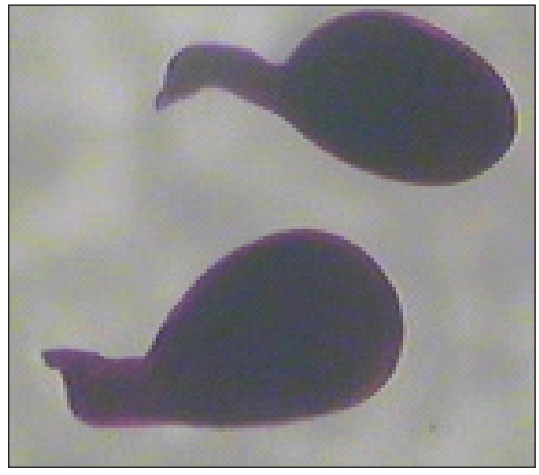
Pomegranate growers of Tamil Nadu many times encounter yellowing of leaves that wither just on shaking the twigs, with stunting and branch wise drying of plants, leading to less productivity and fruit quality. Such trees when investigated by assaying soil and root samples were always found to be severely infested with root-knot nematodes. As not many basic studies are available on pomegranate nematode infestation, studies were taken up to record the various plant parasitic nematodes associated with pomegranate, pathogenicity level of root knot nematode, histopathological changes in root sections due to root knot nematode and management using biological agents in such nematode infested fields.

MATERIALS AND METHODS

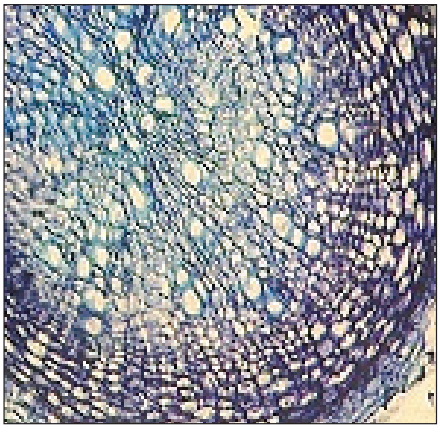
Soil samples were collected from the tree rhizosphere of nematode infested pomegranate trees of variety Bhagwa at a depth of 20-30 cm and at a distance of 120 cm from the trunk. From each locality, five trees were selected randomly and from each tree, three samples were collected. The soil samples were mixed thoroughly and a composite sample of 200cc was taken in polythene bags and sealed tightly with label for processing. The nematodes were extracted by Cobb's sieving and decantation method, followed by modified Baermann's funnel technique (Schinder, 1961). Root samples (5g) collected during survey were stained by acid fuchsin lactophenol method and used for identification of species of the root knot nematode. After clearing with plain lactophenol, the adult females were excised randomly from the stained roots. Ten adult females were selected

Root knot nematode, *Meloidogyne incognita* predisposed wilt in pomegranate plants

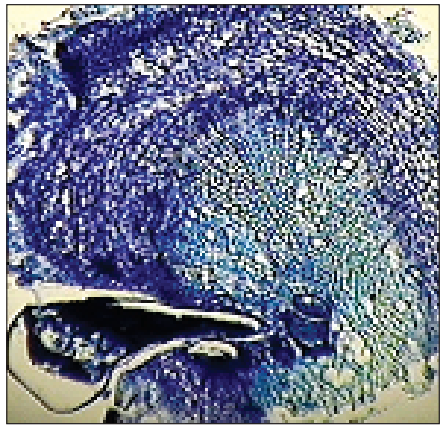
Root knot infested pomegranate roots with minute galls



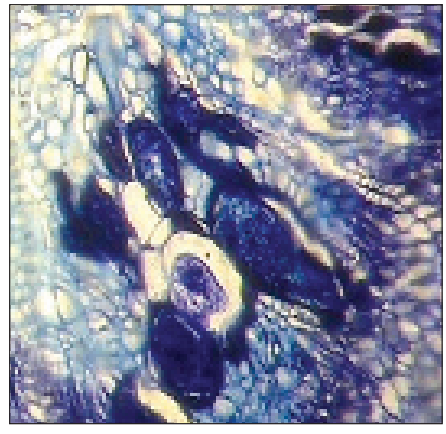
Root knot infested pomegranate roots with minute galls



Healthy section of pomegranate root



Root knot nematode female seen on a section of infested pomegranate root



Giant cells and hypertrophied nuclei around the root knot female that serve as feeding cells

Plate 1. Pictures depicting histopathological studies

randomly and used for species identification by posterior cuticular pattern (PCP) variations (Hartman and Sasser, 1985). The data collected on total nematode population involving soil (200 cc) and root (5g) populations from different locations of pomegranate were subjected to community analysis *viz.,* Absolute and Relative frequency (AF, RF), Absolute and Relative density (AD, RD) and Prominence value (PV) using the following formulae of Norton (1978).

$$\text{Absolute frequency} = \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Sum of frequencies of all species}} \times 100$$

$$\text{Relative density} = \frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$$

$$\text{Absolute density} = \frac{\text{Number of individuals of a species in a sample}}{\text{Volume or mass or units of the sample}} \times 100$$

Prominence Value =

$$\frac{\text{Absolute density x } \sqrt{\text{Absolute frequency}}}{\text{Volume or mass or units of the sample}} \times 100$$

ii. Pathogenicity studies

To establish the pathogenicity of root knot nematode, *M. incognita* in pomegranate (var.Bhagwa), five kg sterile earthen pots containing tissue culture plants in a sterile pot mixture were inoculated with J_2 of *M. incognita* (taken from hatched out egg masses from pure culture maintained under glasshouse conditions) as per the treatment schedule comprising of 10, 100, 1000, 10000 juveniles in 10 ml suspension, through 3cm deep holes near the rhizosphere. In addition, control plants were maintained for comparison. Pots were arranged in completely randomized block design (CRBD) with four replications each. After 90 days, the experiment was terminated by recording the observations on plant growth and nematode reproduction characters.

iii. Histopathological studies

The infested roots were collected and washed gently in tap water and cut into small bits (0.5 to 1.0 cm length); then fixed and dehydrated through ethyl alcohol series followed by embedding the processed root bits in paraffin wax. Root sections were made at 10 µ with the aid of spencer's rotary hand microtome and stained with safranin, counter stained with fast green and finally mounted in D.P.X mountant.

iv. Management studies

Field trials in pomegranate var. Bhagwa plants spaced at 6 x 8 feet, were conducted in farmers' field at Theethipalayam, Coimbatore district under irrigated conditions with the following treatments each replicated thrice in a randomized block design. The treatments included T_1 = *Bacillus subtilis* @ 15 g/plant (cfu 2 x 10^6); T_2 = *Pseudomonas fluorescens* @15 g/plant(cfu 2 x 10^6); T_3 = *Trichoderma harzianum* @15 g/plant(cfu 2x 10^6); T_4 = *Purpureocillium lilacinim*@ 15 g/plant (cfu 2 x 10^6); T_5= *B. subtilis* @ 15 g/plant + Carbendazim @ 2 g/l of water (drenching/ plant); T_6= *P.fluorescens* @15 g/ plant + Carbendazim @ 2 g/l of water (drenching /plant); T_7 = Carbendazim @ 2 g/l of water (drenching per plant); T_8 = Phorate 10G @ 15 g/m^2 (Standard check) and T_9 = Untreated control. Trial was initiated after assessing the initial nematode population which was more than one nematode per gram of soil. Treatments were applied around root zone. The bioagents treatments were given once in three months mixed with FYM and chemicals were one-time application. Nematode counts were taken at three months' interval in 200cc of soil and 5g of root.

RESULTS AND DISCUSSION

i. Community analysis

Random survey for the occurrence and distribution of nematodes was carried out in major pomegranate growing districts of Tamil Nadu *viz.,* Erode, Coimbatore and Dindigul districts. The species of various genera were fixed by drawing Camera Lucida diagrams and by using taxonomic keys. The other genera associated with pomegranate were spiral nematode, *Helicotylenchus multicinctus,* reniform nematode, *Rotylenchulus reniformis,* lesion nematode, *Pratylenchus coffeae* and needle nematode, *Xiphinima index*. Among the nematodes associated with pomegranate, the root knot nematode, *M. incognita* was observed to cause severe damage by causing root galls. Adult females of root knot nematode excised at random from the roots of pomegranate were identified based on the posterior cuticular pattern and the species fixed based on the posterior cuticular pattern. The perineal pattern of twenty root knot nematode females showed the typical characters of high squarish dorsal arch containing distinct whorl in the tail terminus, smooth/wavy striae, forks in the striae at the lateral sides. Hence the nematode was identified as *M. incognita* based on the conventional method using PCP and head regions of juveniles.

The community analysis of nematodes associated with pomegranate inferred that the relative frequency, relative density and prominence value were the highest for root knot nematode followed by reniform nematode which is in confirmation with the findings of Khan, 2005 who reported similar species of nematodes from rhizosphere soils and roots of pomegranate from Baluchistan in Pakistan. Shelke and Darekar (2001) reported the three most common nematode genera found associated with pomegranate in India *viz., H. multicinctus, X. index* and *M. incognita*. The results of the survey were also in accordance with the findings of Lugo who surveyed for the nematodes associated with the pomegranate and other fruit crops and found that *Meloidogyne* spp., *Helicotylenchus* spp. and *R. reniformis* as predominant nematodes. Sudheer *et al*. (2008) reported the occurrence of *Helicotylenchus* spp., *Xiphinema* spp. and *M. incognita* on pomegranate in Andhra Pradesh. Illangovan and Poornima (2017) had concluded that though several plant parasitic nematodes are associated with pomegranate crop, the root knot nematode, *M. incognita* was found to be most commonly occurring as per the community analysis and the probable cause for predisposing plants

Table 1.Community analysis of phytonematodes associated with pomegranate var. Bhagwa

Block	Community analysis	Nematode population				
		M.i	***H. m***	***P. c***	***X. i***	***R. r***
Erode						
Sivagiri	AF	100	96	82	70	100
	RF	24	20	10	16	18
	AD	1892.5	462.8	395.3	217.8	1878.4
	RD	65.4	13.4	12.7	11.0	45.3
	PV	320	60	40	44	217
Nallasellipalayam	AF	100	84	74	72	100
	RF	25	20	13	12	20
	AD	1830.4	349.5	237.1	214.3	1898.2
	RD	63.5	20.2	18.4	13.5	72.4
	PV	318	90	66	47	324
Coimbatore						
Thondamuthur	AF	100	88	80	68	100
	RF	29	22	15	19	18
	AD	1738.0	418.3	211.6	118.4	1776.0
	RD	57.2	24.6	16.8	8.3	68.4
	PV	308	115	65	36	290
Theethipalayam	AF	100	100	76	80	100
	RF	25	18	18	19	16
	AD	1843.0	408.3	256.2	96.4	1830.6
	RD	68.9	24.6	20.8	11.3	68.2
	PV	345	104	88	49	273
Dindugal						
Palani	AF	100	96	72	64	100
	RF	23	20	11	18	16
	AD	1642.0	312.6	176.4	308.6	1830.6
	RD	49.8	18.7	13.5	17.4	64.7
	PV	238	84	45	74	259

*AF=Absolute Frequency (%); * RF=Relative Frequency; *D=Density; PV=Prominence Value

Mi=Meloidogyne incognita; *Hm=Helicotylenchus indicus*; *Pc=Pratylenchus coffeae; Xi=Xiphinema index; Rr=Rotylenchulus reniformis*

to wilt fungus causing yield loss and ultimate death of plants.

ii. Pathogenicity studies

The studies revealed that 10,000 juveniles caused maximum reduction in growth parameters such as length, fresh weight and dry weight of shoot and length, fresh weight and dry weight of root with highest gall index and reproduction factor. Similar findings were observed by Mathi *et al*. (2019) who reported that after six weeks of inoculation, effect of *R. reniformis* on plant growth were visible in infested plants inoculated with 4000 and 8000 fourthe stage juveniles (J_4). In the advanced stage of infestation, leaves showed drying of their margins.

iii. Histopathological studies

Histopathological studies of root knot nematode, *M. incognita* infested pomegranate root sections showed root knot nematode, *M. incognita* females lodged at the pericylce region forming giant cells with denser cytoplasm and multinucleate condition. It is known that all the *Meloidogyne* species irrespective of their host range induce giant cells of same shape and size (Blok *et al.,* 1997). Guava root sections infested with root knot nematode, *M. enterolobii* revealed that the numbers of females were higher (in clusters in nature of about more than 40-50) compared to other root knot nematodes parasitizing crop plants as also the number of giant cells and their size (Ashokkumar *et al*., 2019). Deformation and blockage of vascular tissue at feeding sites limit translocation of different nutrients and water resulting in suppression of plant growth and adversely affecting yield (Hussey and Williamsons, 1997). Histopathological studies of apple roots naturally infested by root knot nematode, *M. incognita* showed that the second-stage juveniles after penetration entered into cortex and moved along the cortical layer of the cells. Disruption of cortical tissue was observed and a number of cells were present with broken walls. Numerous giant cells were also found which were feeding sites for nematodes (Aly Khan *et al.,* 2010). Deformation and blockage of vascular tissue at feeding sites limit translocation of different nutrients and water resulting in suppression of plant growth and adversely affecting yield (Hussey and Williamsons, 1997).

Table 2. Pathogenicity studies of root knot nematode, *M.incognita* on pomegranate var. Bhagwa

Inoculum level	Plant growth characters								
	Shoot			Root			Root gall index	Final population	RF =Pf/ Pi
	Length (cm)	Fresh weight (g)	Dry Weight (g)	Length (cm)	Fresh weight (g)	Dry Weight (g)			
0	43.40[a]	27.53[a]	6.35[a]	32.56[a]	26.83[a]	9.25[a]	0	0	0
10	34.45[b]	28.32[b]	5.39[b]	29.43[a]	23.96[b]	5.43[b]	1.5	2586	258.6
100	32.96[c]	18.86[c]	4.85[c]	22.26[b]	19.66[c]	3.95[c]	2.7	5934	59.34
1000	30.55[d]	16.53[d]	3.92[d]	18.25[b]	16.32[d]	3.44[d]	3.5	10864	10.86
10000	29.53[e]	14.78[d]	3.33[e]	16.48[c]	15.86[d]	2.98[e]	5	14166	1.41
SEd	0.4363	0.1948	0.0512	2.5906	0.2460	0.0536	-	-	-
CD (5 %)	0.9300	0.4152	0.1091	5.6445	0.5243	0.1142	-	-	-

RF=Reproduction Factor; Pf=final population; Pi=Initial population

Table 3. Nematode population in three months' interval (pooled data of two trials)

Sl.No.	Nematode population (200cc soil + 5g root) at 3 months interval							
	3		6		9		12	
	soil	root	soil	root	Soil	Root	Soil	Root
T1	327^{b}	31^{c}	331^{a}	36^{b}	356^{a}	41^{b}	380^{ab}	50^{b}
T2	336^{b}	33^{d}	359^{b}	38^{c}	367^{ab}	45^{c}	400^{b}	60de
T3	338^{b}	32^{cd}	355^{b}	38^{c}	378^{b}	53^{ef}	432^{c}	62^{e}
T4	328^{b}	28^{b}	347^{ab}	32^{a}	365^{ab}	37^{a}	376^{a}	45^{a}
T5	336^{b}	38^{f}	362^{b}	40^{d}	380^{b}	55^{f}	466^{d}	50^{b}
T6	340^{b}	35^{e}	362^{b}	43^{e}	377^{b}	50^{d}	444^{cd}	57^{cd}
T7	379^{c}	36^{e}	400^{c}	47^{f}	433^{d}	53^{ef}	498^{e}	58^{cd}
T8	301^{a}	23^{a}	333^{a}	35^{b}	400^{c}	40^{b}	444^{cd}	56^{c}
T9	380^{c}	35^{e}	402^{c}	48^{f}	456^{e}	52de	533^{f}	60de
SEd	0.22	0.05	0.21	0.07	0.19	0.06	0.28	0.09
CD at 5%	0.47	0.11	0.45	0.15	0.41	0.14	0.59	0.21

Table 4. Plants showing wilting and revival symptoms and yield parameters (pooled data of two trials)

Treatment	RKI	Number of plants showing		Yield t/ha
		Wilting symptoms	Revival	
T1	3	3	1	15.03^{a}
T2	4	-	-	13.07^{c}
T3	4	1	-	13.89^{bc}
T4	2	3	2	15.28^{a}
T5	5	-	-	13.71^{bc}
T6	3	-	-	13.04^{c}
T7	5	4	4	13.37^{c}
T8	3	-	-	14.42^{ab}
T9	5	4	-	12.10^{d}
SEd	-	-	-	0.06
CD 0.05	-	-	-	0.12

iv. Management studies

Among the bioagents applied, both *Purpureocillium lilacinum* and *Bacillus subtilis* @ 15 g/plant (cfu 2 x 10^6) applied thrice every three months interval along with FYM were found equally effective in reducing population of root knot nematode, *Meloidogyne incognita* and in increasing yield by 26.28% and 24.21% respectively. The fungal biocontrol agent, *Paecilomyces lilacinus* strain 251 (PL251), was evaluated for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato. A pre-planting soil treatment reduced root galling by 66%, number of egg masses by 74% and the final nematode population in the roots by 71% compared to the inoculated control (Kiewnick and Sikora, (2006). Khan and Saxena (1997) reported that the integration of oil-cakes (except mahua-cake), bone and horn meals with *P. lilacinus*, resulted in increased plant growth and reduced population build-up of nematodes and root gallings. The organic amendments increased the parasitism of *P. lilacinus* on root-knot nematodes. Syed and Khan (2006) found in their studies that the best protection against *M. incognita* was observed on the integration of organic additives with *P. lilacinus,* which resulted in increased plant growth and reduced population build-up of nematodes and root galling (Tables 3 and 4).

Swarnakumari and Kalaiarasan (2017) observed that the vegetative hyphae of *Purpureocillium lilacinum* first entered into the gelatinous matrix and then got attached on the surface of nematode egg in 24 h. On the second day, approsorium appeared and penetration peg formed below the approsorium. Mycelial growth was observed on the egg surface at 72 h after conidial inoculation. The egg was completely colonized on the 4th day after inoculation. Fungal structure phialids which were flask shaped with swollen base emerged out from the egg on 7th day after inoculation after which there was complete stopping of embryonic development. Eggs remained in the gastrula stage with no further cell division. El-Nagdi and Abd-El-Khair (2019) recorded that *Bacillus* sp. (40 ml) significantly reduced the J_2 in soil and galls and egg masses in roots, while *B. pumilus* (40 ml) significantly reduced the J_2 in roots. The nematicidal activity of *Bacillus* spp. was increased by increasing the applied dose.

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