

Screening and histological characterization of guava (*Psidium guajava* L.) cultivars against root knot nematode, *Meloidogyne enterolobii*

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ABSTRACT: Surveys were carried out in major guava growing districts of Tamil Nadu in India for incidence of guava root knot nematode, *Meloidogyne enterolobii*. Fifteen cultivars *viz.*, Alahabad Safeda, Lucknow-46, Thailand Red, Banaras, Lucknow-49, Mini Guava, Local, Beetroot Guava, Trichy-1, Hisar Lalit, Taiwan Guava, Chitidar, White seedless, Red seedless, and Panneer guava were collected from various nurseries and farmer fields for screening. The screening results showed that Mini Guava(4.0), Local(4.0), White seedless(3.6), Red seedless(3.6), Panneer guava(3.6) were susceptible to *M. enterolobii*, based on gall index while other cultivars were highly susceptible. Root exudate was collected from 15 cultivars and tested the hatching ability of eggs and second stage juvenile (J₂s) mortality rate. The root exudate increased the egg hatching ability and decrease the juvenile mortality rate. The increase in hatching ability and decrease in mortality rate were directly proportional to time and concentration. Moreover, the histopathological response of *M. enterolobii* infested roots showed complete damage of the epidermis, cortex, endodermis, pericycle, xylem and phloem cells of roots and different stages of *M. enterolobii* were exist in clusters.

Keywords: Guava, Meloidogyne enterolobii, screening, root exudates, histopathology

INTRODUCTION

Guava (Psidium guajava L.) also called as 'poor man's apple is one of the important commercial fruits in India, occupying an area of 2.03 lakh hectares with an annual production of 22.7 lakhs MT. Major guava growing states in India are Uttar Pradesh, Bihar, West Bengal, Maharashtra, Chattisgarh, Tamil Nadu, Karnataka, Madhya Pradesh, Gujarat and Andhra Pradesh. Recently, in Tamil Nadu, M. enterolobii was detected for the first time on guava (Poornima et al., 2016). Considering the risk of introduction and dissemination of the pest, Melodiogyne enterolobii was recently added in EPPO A2 list (No. 361, OEPP/EPPO Bulletin, 2014). M. enterolobii has been considered as a matter of grave concern, because it has been spreading rapidly and makes the cultivation of guava unviable in heavily infested areas (Carneiro et al., 2007). The root knot nematode generally occurs in polyspecific communities, interacts in a dynamic way with the host plant, the environment and the other organisms present in the rhizosphere. The present study was taken up to attempt possibility of getting a resistant variety against this guava root knot nematode, M. enterolobii, which could be used as a rootstock for producing nematode free guava grafts.

Histological studies on fruit trees roots infested

with plant nematodes have been conducted from time to time (Khan *et al.*, 2004; 2007, Sayed *et al.*, 2008). Samad *et al.* (2012) reported histological changes in guava seedling roots infested by *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949). However, this paper presents the histological changes in guava roots of seedlings infested with guava root knot nematode, *M.enterolobii*.

MATERIALS AND METHODS

Field Surveys

Surveys were conducted in the major guava growing areas of different districts in Tamil Nadu viz., Coimbatore, Erode, Theni, Madurai, Krishnagiri, Dharmapuri, Villupuram, Thiruvannamalai and Dindigul (Table 1). Soil (200gm) along with feeder roots was collected and put into the polythene bags and tied with rubber band to check evaporation. Supporting data regarding coordinates, crop, variety, date of collection was tagged with the bag. The samples were then brought to the nematology laboratory (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India) for further processing. For extraction of nematodes from the soil, Cobb's decanting and sieving followed by Baermann's funnel technique (Schindler, 1961) was followed by using a series of sieves (20, 60, 150 and 350 mesh). After

24 hours the nematode suspension was collected and examined under a stereo zoom binocular microscope.

For extraction of nematodes from root, Acid fuchsin - Lactophenol method (Bybd et al., 1983) was followed. Root samples of known quantity were taken, thoroughly washed and soaked in 3 % sodium hypochlorite (NaOCl) for 2 min and then washed in distilled water resulting that the samples were free from residue. The washed roots were then added into a small beaker containing boiling acid fuchsin + lactophenol solution (stock solution was prepared by dissolving 1g of acid fuchsin stain in 100 ml of water, from this stock solution 5 ml was mixed in 100 ml lactophenol) and heaedt for three min or until the formation of bubbles in the solution (Bybd et al., 1983). These stained roots were washed in tap water to remove the excess stain and transferred to a beaker containing plain lactophenol solution and allowed to be undisturbed for 12 h for destaining. The stained adult females of M.enterolobii nematode on roots were observed and counted under stereoscopic binocular microscope.

Maintenance of pure culture of root knot nematode, *M. Enterolobii*

Pure culture of root knot nematode, *M. enterolobii* is required for conducting further studies and was cultured on guava (L-49) variety in the glasshouse conditions. The egg masses were collected from infested guava plants of a farmer's field at Ayakudi (Dindigul district). (10°26'56.11"N77°31'15.38"E).Identification of species

of root knot nematode was done by observing their posterior cuticular pattern (PCP). Then, the collected egg masses were allowed for hatching with proper aeration for five days. Guava seedlings collected froma private nursery in Ayakudi were planted in five kg capacity of earthen pots filled with sterilized pot mixture (Red soil: Sand: FYM – 2:2:1). The hatched out juveniles (J_2) of *M. enterolobii* were inoculated in the rhizosphere region of the potted guava plants @ one J_2 /g of soil. Nematodes were allowed for further multiplication and the egg masses required for the experiments were collected from the pure culture.

Screening of guava cultivars against root knot nematode, M. enterolobii

The guava cultivars viz., Alahabad Safeda, Lucknow-46, Thailand Red, Banaras, Lucknow-49, Mini Guava, Local, Beetroot Guava, Trichy-1, Hisar Lalit, Taiwan Guava, Chitidar, White seedless, Red seedless, Panneer guava were screened against M.enterolobii at different inoculum levels viz., one J_2 and two J_2 per gram of sterilized soil with three replications. Final soil nematode population and gall index were recorded at 65 days after inoculation and the reproduction factor (RF) calculated

Indexing: Nematode reproduction was assessed by calculating the Oostenbrink (1966) reproduction factor (RF) = Pf/Pi (as final population (Pf) / initial population (Pi). If RF is more than 1 (Susceptible); RF less than 1 (Resistant) (Rodriguez- kabana and Morgan Jones, 1988).

Table 1. Classification of genotypes based on gall index

No of galls	Gall index	Reaction
0	1	Highly resistant (HR)
1-10	2	Resistant (R)
11-30	3	Moderately resistant (MR)
31-100	4	Susceptible (S)
101 and above	5	Highly susceptible (HS)
		/II 4 1 100 <i>5</i>

(Hartman and sasser, 1985)

Collection of root exudates

The starter cultures of *M.enterolobii* were transferred to mud pots containing sterilized pot mixture. The guava seedlings were transplanted to the mud pots as one plant/pot. Regular watering was done and these plants were maintained under glasshouse conditions (temperature-26-30 °C, RH-42%). The root exudates were collected after 60 days of transplanting. The regular watering was stopped for two days and on the third day 500 ml of water was poured to each plant and the root exudates from the pots were collected in a pan placed underneath

the pots with plants. The root exudates were collected in a glass beaker from the above plants. For getting a clear suspension, the root exudates were filtered through a Whatmann No. 1 filter paper and stored in a conical flask under room temperature (30 $^{\circ}$ C). Stored root exudates were used for further hatching and mortality studies.

Effect of guava root exudates on hatching of *M.enterolobii* eggs

The root knot nematode egg masses were collected. Small petri dish of 5 cm dia was used for hatching test. Petri dishes were supplied with 1 ml of particular root exudates and distilled water is used as a control as per the treatment. Each petri dish was placed with one egg mass and incubated under room temperature. Observations were recorded at 12h, 24h, 48h and 72h interval with the concentrations of 25,50,75 and 100 per cent. Then, the number of hatched out juveniles were counted. After 3rd day the number of unhatched eggs was counted and hatching percentage was calculated. The experiment was conducted in a Completely Randomized Block Design with five treatments and five replications. Hatching percentage= No. of hatched juveniles / Total no. of eggs× 100.

Effect of guava root exudates on juveniles of *M.enterolobii*

Petri dishes were supplied with 1 ml of root exudates and distilled water is used as a control as per the treatment. 100 freshly hatched juveniles (J_2) were placed in each petri dish and incubated at room temperature. Observations were recorded at 12h, 24h,48h and 72h interval with the concentrations of 25,50,75 and 100 per cent. Thus, the mortality rate of M. enterolobii juveniles (number of dead juveniles) was calculated. The experiment was conducted in a Completely Randomized Block Design with five treatments and five replications.

Study of histopathological changes in the roots infested by *M.enterolobii* in guava

Histopathological studies were done with guava plants infested with root knot nematode M.enterolobii 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. And the sectioning was made at $10~\mu$ with the aid of spencer's rotary hand microtome. Those sections were stained with safranin, counter stained with fast green and finally mounted in D.P.X mountant (Jenson, 1962).

RESULTS

Nematode distribution

Surveys were conducted in guava growing areas of Tamil Nadu *viz.*, Coimbatore, Erode, Theni, Madurai, Krishnagiri, Dharmapuri, Villupuram, Thiruvannamalai and Dindigul. It was observed that root knot nematode, *M.enterolobii* infested guava orchards in each of the district surveyed. They had symptoms such as bronzing of leaves with marginal necrosis, simple and compound galls in the roots and browning of younger and older leaves, results in wilting of plants. Wherever it was combined with fungal infection, the plants showed

sudden death of plants.

Screening of guava cultivars against M.enterolobii

Fifteen cultivarsof guava were screened (Plate2.) for *M.enterolobii*, by means of gall index (GI) from 0 (with 0 no of gall) to 5 (with 101 and above galls), 65 days after inoculation of infective juveniles (1J₂/g of soil and 2J₂/g of soil (Table 2). Five of them showed susceptible reaction with a GI range of 3.6 to 5.0; five others showed susceptibility and ten cultivars showed highly susceptibility under artificially inoculated conditions. The RF (Reproduction Factor) was higher in Thailand Red (1.73), Hisar Lalit (1.72) and Taiwan Guava (1.83) compared toLucknow-49 (1.38), Taiwan Guava (1.39) and Red Seedless (1.37) which were comparatively lesser (Table 3).

Effects of guava root exudates on M. Enterolobii

In this study, root exudates obtained from 15 guava cultivars (R1 to R15) were found to increase the hatching ability of M. enterolobii eggs and decrease the mortality rate of J₂ (Second stage juveniles). The result obtained from root exudates of 15 guava root stocks at different time intervals (12,s24,48 and 72 hours) were studied. In 12 hours, all 15 root exudates showed gradual increase in their hatching whereas hatching rate was very high in R3 (25%-25.60, 50%-31.20,75%-40.00,100%-49.10) R11 (25%-24.20,50%-30.10,75%-43.60,100%-52.10) Mortality rate was very low in R2 (25%compared 1.80,50%-2.00,75%-2.40,100%-2.80) control (Figure 1).In 24 hours, all 15 root exudates showed gradual increase in their hatching whereas hatching rate was very high in R11 (25%-36.60,50%-38.40,75%-49.00,100%-57.60) Mortality rate was very low in R2 (25%-2.10,50%-2.60,75%-3.00,100%-3.20) compared to control (Figure 1). In 48 hours, all 15 root exudates showed gradual increase in their hatching whereas hatching rate was very high in R12 (25%-42.00,50%-50.10,75%-61.20,100%-69.10) and R13 (25%-42.00,50% -52.00,75%-60.10,100%-69.20) mortality rate is very low in R5 (25%-3.40,50%-3.80,75%-4.10,100%-4.60 compared to control (Figure 1).In 72 hours all 15 root exudates shows gradual increase in their hatching whereas hatching rate is very high in R2 (25%-52.00,50%-56.20,75%-61.10,100%-75.00) (25%-49.10,50%-58.60,75%-69.20,100%-76.00) mortality rate is very low in R13 (25%-5.20,50%-9.40,75%-12.80,100%-16.20 compared to control (Figure 1).

Histopathological characterization of guava root infested by root knot nematode, *M.enterolobii*

The root-knot nematode is soil-borne; only the

Table 2. Occurrence of root knot nematode, M.enterolobii in Tamil Nadu

Location	Co ordinates			_	Nematode population in roots			Nematode Population
	Lattitude o(E)	Longitude o(N)	Cultivars	Previous crop root	No. egg mass/5g	No. females/gall	No. eggs/egg mass	in soil 200 cc
THENI								
Uthamapalayam	9.8712518	77.3253846	Banaras	Banana	28	21	125	84
Uthamapalayam	9.872479	77.327525	Banaras	Banana	31	18	109	82
Uthamapalayam	9.875763	77.328210	L -4 9	Brinjal	29	14	112	99
Chinnamanoor	9.8126624	77.378182	L-49	Tomato	27	20	140	77
Cumbam	9.727779	77.313938	Arka Kiran	Banana	24	16	102	72
				Total	139	89	588	414
MADURAI								
Melur	9.924079	78.304369	Taiwan	Banana	17	14	97	89
Melur	9.922135	78.304984	Taiwan	Banana	16	13	84	77
Melur	9.921592	78.305817	Taiwan	Banana	27	9	87	79
				Total	60	36	268	245
DHARMAPURI								
Nekkunthi	12.075610	77.979281	L-46	Groundnut	48	19	109	94
Nallampalli	12.122567	78.016868	Allahabad Safeda	Tapioca	54	24	109	67
Dharmapuri	12.201561	78.220453	L-49	Paddy	43	16	112	76
Mookanur	12.146709	78.264076	Hisar Lalit	Papaya	51	27	124	68
				Total	196	86	454	305
KRISHNAGIRI								
Kaveripattinam.	12.408927	78.190921	Taiwan Pink	Paddy	48	24	98	83
Kaveripattinam.	12.410292	78.191404	Taiwan Pink	Paddy	53	21	80	71
Kaveripattinam.	12.408582	78.190008	Taiwan Pink	Paddy	41	26	92	69
Kaveripattinam.	12.4099529	78.1905395	Taiwan Pink	Paddy	38	19	101	53
				Total	180	90	371	276
VILLUPURAM								
Ulundurpet	11.831595	79.474487	Hisar Lalit	Crossandra	29	12	79	43
Ulundurpet	11.831859	79.473744	Hisar Lalit	Crossandra	24	08	80	66
				Total	53	20	159	109
DINDIGUL								
Palani	10.4968284	77.5747435	L-49	Onion	47	34	121	74
Palani	10.497520	77.574006	L-49	Onion	53	29	109	82
Amarapoondi	10.4950950	77.5715260	Banaras	Brinjal	51	31	116	58
Amarapoondi	10.494432	77.572025	Banaras	Brinjal	56	36	121	49
				Total	207	130	467	263
COIMBATORE								
Thondamuthur	11.0250481	76.8839245	L-49	Cumbu	20	28	92	63
Thondamuthur	11.024140	76.884562	L-49	Cumbu	24	24	82	54
Thondamuthur	11.024597	76.884146	L-49	Cumbu	16	27	89	43
Thondamuthur	11.025029	76.884426	L-49	Cumbu	21	31	94	59
Karmadai	11.277816	76.933085	L-49	Coconut	46	24	95	73
				Total	127	134	452	292
THIRUVANNAM								
Avoor	12.1468353	79.2209262	L-49	Sugarcane	24	26	94	68
Avoor	12.145752	79.220646	L-49	Sugarcane	27	37	93	74
Avoor	12.145100	79.220621	L-49	Sugarcane	31	31	102	86
Avoor	12.145453	79.221086	L-49	Sugarcane	29	35	104	89
				Total	111	129	393	317
ERODE								
Anthiyur	11.6978290	77.6641880	L-46	Turmeric	19	16	89	78
Anthiyur	11.698560	77.666926	L-46	Turmeric	21	13	107	61
Anthiyur	11.698146	77.666014	L-46	Turmeric	19	17	99	56
Anthiyur	11.697035	77.667659	L-46	Turmeric	24	19	109	72
				Total	83	65	404	267

vermiform juveniles are present in soil that invade the roots and modify the vascular tissues (xylem and phloem cells) into "giant cells" that nurse the developing juveniles. Soon after invasion and after establishing the "feeding relationship" with its plant host, the nematode becomes sedentary and assumes swollen shape. Most of the invaded juveniles become sac-like females, while some of them become vermiform males. The adult males leave the roots and emerge into soil. The females draw nutrition flowing through the conducting vessels via giant cells and start laying eggs (200-400 eggs per female) on the root surface nested in masses. Sometimes, the egg masses are formed inside the roots themselves (Poornima and Walia, 2017).

In the case of guava root knot nematode, *M. enterolobii*, the number of females may be few in simple galls and many in compound galls, with long necks with a bulb like posterior, crowding to reach the pericycle area. This enormous crowding seems to cause chocking of the stealar region which ultimately results in disruption of the vascular bundles and its function.

This was clearly seen in the root sections taken using microtomy. The uninfested roots sections reveal intact epidermis, cortex, xylem and phloem cells devoid of any damage or cavities (Plate 3 (A)). While, the guava root knot nematode infested roots sections showed complete disruption of the epidermis, cortex, endodermis, pericycle, xylem and phloem cells. The sections are occupied by clusters of adult females (sometimes even upto 60 per gall) of M.enterolobii and nearly one fourth of the sectioned area is occupied with giant cells of various sizes which are found more in the deeper layers of cortical region. The nematodes found in the intercellular spaces of the cortical region were found straining their necks to reach the pericycle. The conducting vessels were observed to be chocked and pushed to the periphery of the root (C). The giant cells surrounding the nematode head were in groups, each with 8-10 cells of varying sizes, with granular cytoplasm and multiple nuclei as a result of hyperplasia and hypertrophy (D). The surrounding cortical cells were completely damaged (E). Matured females of M.enterolobii were present in the pericycle region (F). The cortical cells showed granulations and the cell organelles were condensed at the centre of the cells. Large cavities were seen in the cortex and many of the cells were distorted.

DISCUSSION

Nematode distribution

The root knot nematode, widely infest the guava roots in orchards growing in and around Aligarh district of Uttar Pradhesh. Nematode infested plant exhibits stunting, yellowing, dieback and patchy growth. However, the root system showed numerous small to big sized galls. The investigation has clearly indicated that the association of root knot nematode, M.incognita with guava was highly pathogenic in nature (Ansari and Khan, 2012). But in case of Tamil Nadu, M.enterolobii cause severe yield loss to guava orchards, first record from India (Poornima et al. 2016). The M. mayaguensis has been reported in some states of Brazil causing severe damage on commercial guava (Carneiroet al., 2011). Almeida et al., (2009) reported that M.enterolobii (syn M.mayaguensis) was a polyphagous plant parasitic nematode causing severe damage in several plant species in Brazil. This study was conducted in major guava growing districts of Tamil Nadu. During this survey M.enterolobii infestation was observed in guava from all the major guava growing districts. Gomes et al., (2012) observed that guava decline was a complex disease which attains parasitism by M.enterolobii results in yellowing, wilting, scorching of leaf margins and leaf drop, yield reduction and plant death within few months. Similar kinds of symptoms were also observed during survey.

Screening of guava cultivars used as cultivars against root knot nematode, *M.enterolobii*

This is the first study on screening of different guava cultivars against root knot nematode, *M.enterolobii*, used as cultivars that are now commercially growing in different parts of Tamil Nadu and also in India. The uninfested guava cultivars were collected in various nurseries and in farmers field and artificially inoculated with the *M.enterolobii* J₂s. The observations shows that all the cultivars were susceptible to *M.enterolobii* and some of them are highly susceptible. Thus, concluded that all commercially cultivating *Psidium* spp were susceptible to *M.enterolobii*. Ferris *et al.*,(2012) reported that based on their rooting capability and horticultural characteristics, 200 candidates were selected from 5,000 progeny of commercial grape root stocks and wild grape species that exhibited resistance to nematodes.

Effects of root exudates on M. Enterolobii

Plant guava roots release numerous chemical compounds including amino acids, complex polysaccharides, protein and smaller, volatile, lipophilic molecules. All of these will directly and indirectly influence the soil organisms, such as the nematodes (Bais et al., 2006). Extensive studies have shown that root exudates influence the egg hatching of nematodes. The exudate from nematode resistant plants have been observed to suppress the rate of egg hatching ability while exudates from nematode susceptible plants may stimulate egg hatching ability (Yang et al., 2016). In this

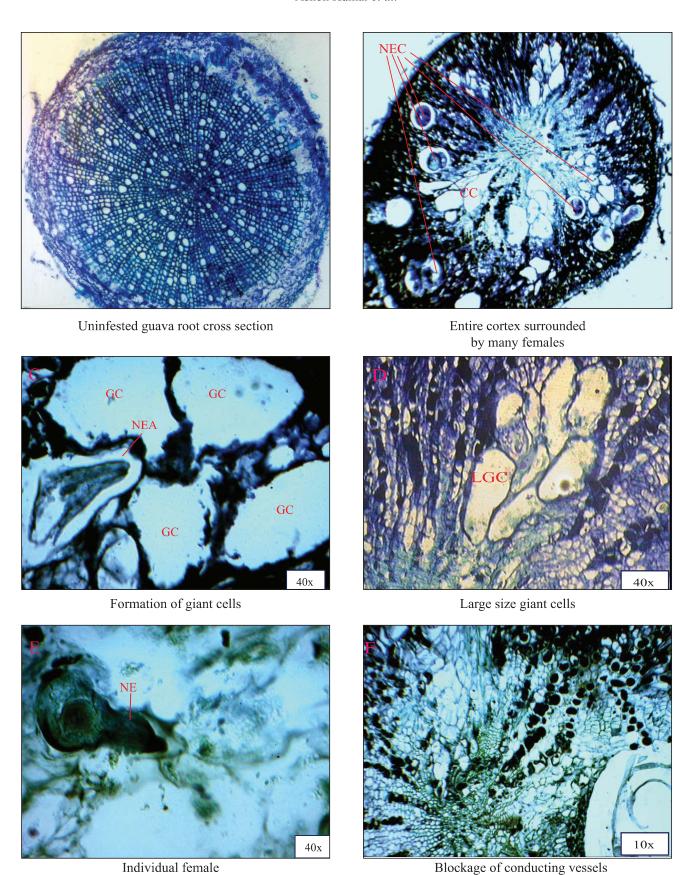


Plate 1: Histopathological changes in guava root infested by root knot nematode, *M.enterolobii* NE=Nematode, NEC=Nematode clusters,NEA=Anterior portion of nematode, GC=Giant cell, LGC=Large sized giant cell

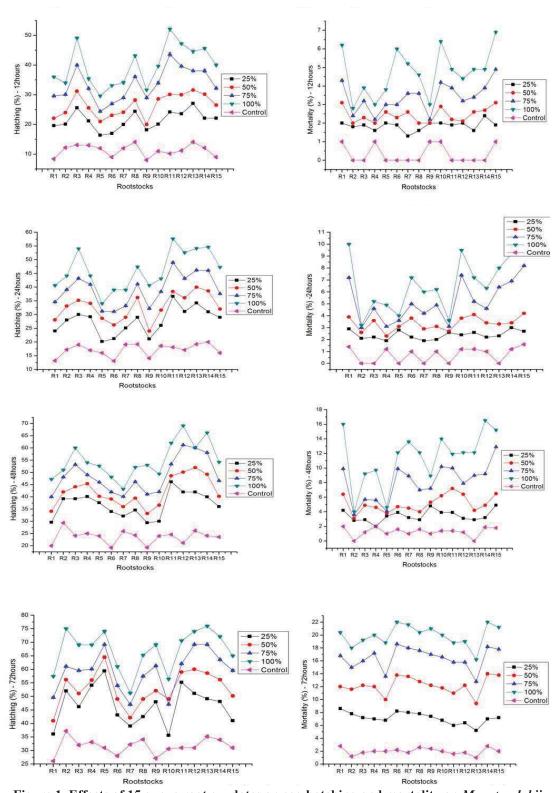


Figure 1. Effects of 15 guava root exudates on egg hatching and mortality on *M. enterolobii* R1.Alahabad Safeda R2.Lucknow-46 R3.Thailand Red R4.Banaras R5.Lucknow-49 R6.Mini Guava R7.Local R8.Beetroot Guava R9.Trichy-1 R10.Hisar Lalit R11.Taiwan Guava R12.Chitidar R13.White seedless R14.Red seedless R15.Panneer guava

Table 3. Screening of guava root stocks against root knot nematode, M.enterolobii

	1J ₂ /g of soil			2J ₂ /g of soil			
Cultivar	Final population	RF	Gall Index	Final population	RF	Gall Index	
Alahabad Safeda	8041.66	1.61	4.6**	12628.33	1.26	5.0**	
Lucknow-46	7906.66	1.60	4.3**	12610.00	1.26	4.6**	
Thailand Red	8660.00	1.73	5.0**	12865.00	1.28	5.0**	
Banaras	8137.33	1.63	4.3**	12738.00	1.27	4.6**	
ucknow-49	8716.66	1.74	4.6**	13858.33	1.38	4.6**	
⁄Iini Guava	7696.66	1.54	4.0*	12575.00	1.25	4.0*	
ocal	7890.00	1.57	4.0*	12066.66	1.12	4.0*	
Seetroot Guava	8346.66	1.66	4.6**	12935.00	1.29	5.0**	
richy-1	8406.66	1.68	4.6**	12138.33	1.21	5.0**	
Hisar Lalit	8646.66	1.72	4.6**	13166.66	1.31	4.6**	
aiwan Guava	9146.66	1.83	5.0**	13990.00	1.39	4.6**	
Chitidar	8178.33	1.63	4.3**	12660.00	1.26	4.6**	
White Seedless	7995.00	1.59	3.6*	13096.66	1.30	3.6*	
ed Seedless	8275.00	1.65	3.6*	13750.00	1.37	3.6*	
anneer Guava	7235.00	1.44	3.6*	11989.00	1.19	3.6*	

^{**}Highly susceptible * Susceptible

study, egg hatching ability of M.enterolobii was found to be moderately increasing at different time interval at different concentrations. Hence, it is concluded that the egg hatching was directly proportional to the increase in time period and increase in concentration of the root exudates.

$\label{eq:master} \mbox{Histopathological characterization on guava infested} \\ \mbox{by M.enterolobii}$

Di Vito et al., (2004) recorded that the sections of root knot nematode infested pomegranate roots showed the presence of females of root knot nematode, M.incognita at the pericylce region forming giant cells with densercytoplasm and multinucleate condition. The root galls induced by M. incognita, race 1 on spinach is varied in size and location. Generally, large, spherical, regular galls were present on root tips, and these were also present along root axis. In the present study, an attempt was done to study the histopathological differences caused by the root knot nematode, M. enterolobii infesting guava roots. It was clearly seen in the sections that the numbers of females of M.enterolobii 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. The xylem and phloem vessels were completely distorted due to the presence of *M.enterolobii* in clusters, causing chocking of the conducting vessels thereby disrupting the normal function of uptake of water and nutrients by the roots, causing ultimate wilting of the plants.

The studies show that no cultivated guava variety is resistant or tolerant to this nematode as confirmed by artificial inoculation and by root exudates studies. This narrows down the possibility to searching for a wild cultivar or any other Psidium species that could be tried for as resistance source. The histopathological studies conclude that the entry of several second stage juveniles into the guava root that finally become swollen females, disrupt the conducting tissues due to their occupying major space in the cortical regions apart from forming multiple giant cells, pushing the xylem and phloem cells into a corner. This ultimately disrupts the normal functioning of the root in uptake of water and nutrients that could be visible as the several nutritional symptoms on guava leaves like bronzing and yellowing. The root knot nematode, M.enterolobii mostly allows the entry of the fungus, Fusarium spp. that ultimately cause the death of plants.

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