Pest Management in Horticultural Ecosystems
Vol. 25, No.2 pp 252-256 (2019)

Synergistic effect of root knot nematode, *Meloidogyne enterolobii* and *Fusarium oxysporum* in causing guava decline in Tamil Nadu

K. GANESHAN[1], K. POORNIMA[1], P. RENUKADEVI[2] and S. NAKKEERAN[2]

[1]Department of Nematology, [2]Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

E-mail: poornimamurtysista@gmail.com

ABSTRACT: A pot culture study on the interaction of nematode *Meloidogyne enterolobii* and fungus, *Fusarium oxysporum* was carried out with six different treatments. The disease incidence was 100 % in the treatments inoculated with nematodes followed by fungus and simultaneous inoculation of root knot nematode and fungus. Hence, both the nematode and fungus equally contributed to guava decline. The entry paved by *M. enterolobii* through its feeding sites and infection made the guava plants susceptible to *Fusarium* contributing to the incidence of guava decline. It was observed that root knot index was significantly higher in plants inoculated with nematode alone (3.25) followed by nematode inoculation 15 days prior to fungus. These findings pave way to develop strategies in overcoming guava decline.

Keywords: Guava decline, *Meloidogyne enterolobii, Fusarium* fungi, disease complex

INTRODUCTION

Guava (*Psidium guajava* L.), termed as "poor man's apple"is an important commercial fruit in India. Guava is native of tropical America. It was introduced into India in the seventeenth century (Hays, 1970). It is the fifth most important fruit crops in India after mango, banana, citrus and apple with annual production of 36.4 lakhs MT from 26.1 lakh hectares and productivity 13.94/ha (Anonymous, 2018). The guava production is declining due to a disease caused by a complex of parasitic nematode, *Meloidogyne enterolobii* which predispose a fungus, *Fusariumo xysporum* f.sp. *psidii*.The root knot nematode (RKN), *M. enterolobii,*a species not recorded in India so far (Poornima *et al*., 2016), is a polyphaous phytoparasitic nematode attacking woody plants (Rammah and Hirschmann, 1988; Brito*et al*., 2007 and Gomes *et al*., 2008).Root knot nematodes always increase the severity of *Fuasrium* wilt (Atkinson's, 1982).An attempt was taken to study the synergistic effects of RKN and *Fusarium* wilt causing guava decline.

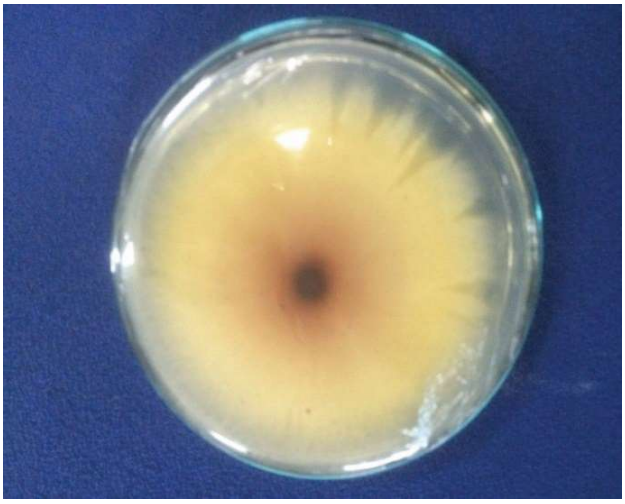
MATERIALS AND METHODS

Maintenance of pure culture of root knot nematode

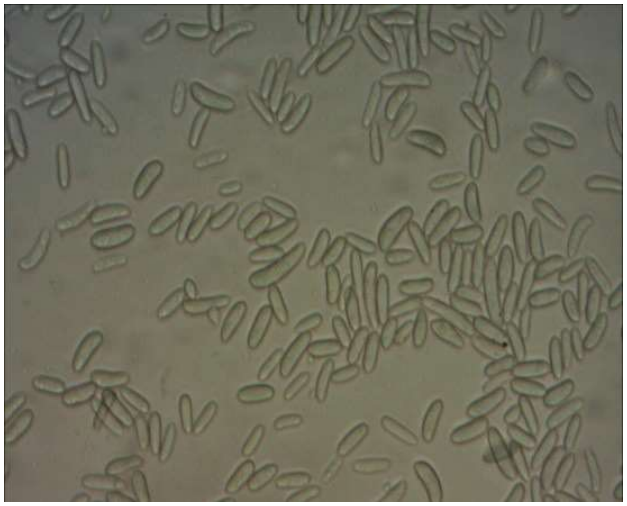
Guava roots with conspicuous galls were selected and washed thoroughly in water and examined for the presence of egg masses under microscope. The egg masses were picked out and kept in a beaker half filled with sterile water and incubated at room temperature with frequent aeration. After three days, the hatched out J_2 of *M. enterolobii* were inoculated on guava as follows. Guava seedlings were transplanted in earthen pots (5kg) containing sterilized pot mixture consisting of sand, red soil and farm yard manure mixed in the ratio of 1:1:2. Root knot nematode, *M. enterolobii* juveniles (J_2) obtained from the egg masses were inoculated @ 2 J_2/g of soil in the rhizosphere region at two weeks after transplanting and covered by sterilized pot mixture soil. The nematodes were multiplied and maintained separately as stock culture in the glasshouse of Nematology, TNAU, Coimbatore. The nematodes required for the experimental purpose were harvested from this culture. A pot culture experiment on nematode-fungus disease complex involving *M. enterolobii* and *F.oxysporum* in guava was conducted in glasshouse.

Preparation of nematode and fungal inoculum

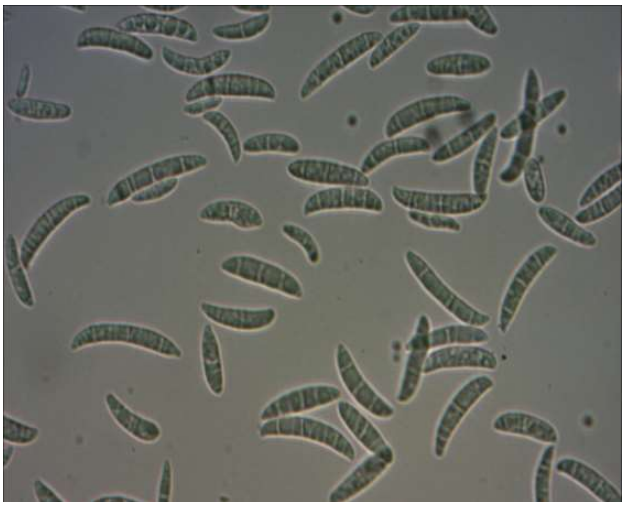
Nematode inoculum was obtained by following the protocols as described in the section above. The fungus inoculum was prepared in sand maize medium. Sand maize medium was prepared with 90g sand, 10g maize meal, 20 ml distilled water. All the components were mixed together uniformly and packed in polythene bags @ 200g / bag. The bags were steam sterilized thrice @ 121°C for 20 min by providing time interval for cooling. The cool sterilized mixture was inoculated with three 9mm mycelia discs of actively growing culture of the guava wilt pathogen. The mixture was incubated for twenty days and the inoculum was adjusted to 10^6cfu/g of the mixture (Plate 1.)



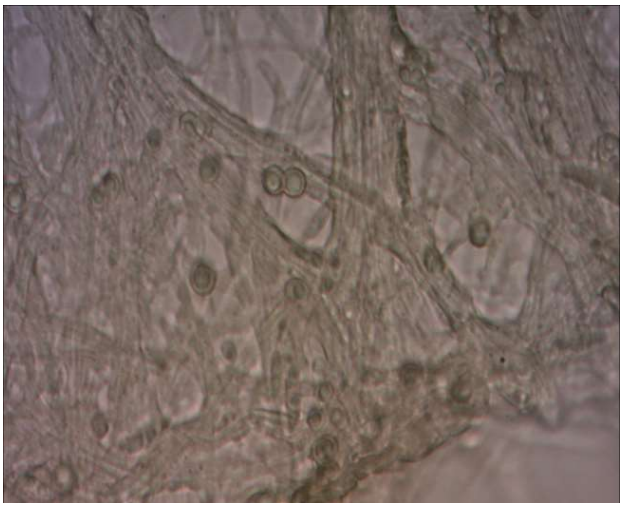
Pure culture of *Fusarium*



Micro conidia



Macro conidia



Micro conidia

Nematode and fungal interaction studies

Nematode fungal interaction studies were executed under glass house conditions. Two months old guava seedlings were transplanted in 5 kg capacity earthen pots containing steam sterilized soil @ one seedling per pot. Twenty days after transplanting, the study nematode and fungus was inoculated in five different treatment combinations. An untreated control was also maintained. The experiments were replicated with four seedlings per treatment. The study nematode was inoculated into the potted seedlings @1 juvenile (J_2)/g of soil. Similarly, 20 days old sand maize inoculum of guava wilt pathogen was applied at 5g/kg soil by removing the soil around the root zone. Treatments as mentioned in Tables 1 and 2 are applied.

After the plants exhibited symptoms, they were uprooted, rinsed free of soil and growth parameters such as root length, shoot length, fresh root and shoot weight, dry root and shoot weight were recorded. Nematode incidence in terms of number of egg masses per 5 g root, eggs per egg mass, adult females per 5 g root, final soil nematode population and gall index were recorded. Similarly percent disease incidence was also recorded as per the formula proposed by Wheeler (1969).

RESULTS

*F. oxysporum*mass multiplied in sand maize medium was inoculated into the sterilized potting mixture containing guava seedlings**.** The symptoms were observed 45 days after inoculation. Among the six treatments, plants inoculated with nematode followed by inoculation with the wilt fungus post 15 days was efficient in establishing wilt as well as the establishment of the nematode. Symptoms included yellowing, drooping and withering of leaves. Within 60 days, the whole plant dried. Cent

Table 1. Effect of combinations of *M. enterolobii* and *F. oxysporum*on the growth parameters of guava under greenhouse conditions

Treatment	Vegetative growth					
	Shoot			Root		
	Length (cm)	Fresh Weight (g)	Dry weight (g)	Length (cm)	Fresh Weight (g)	Dry weight (g)
M. enterolobii alone	31.00[c]	17.00[b]	4.20[c]	18.25[c]	16[b]	3.93[b]
	(25.27)	(40.55)	(27.03)	(39.23)	(36.47)	(52.07)
*F. oxysporum*alone	34.30[d]	26.50[d]	5.35[d]	25.0[d]	22.0[d]	4.00[b]
	(17.34)	(7.51)	(7.38)	(16.64)	(12.52)	(51.22)
M. enterolobii followed by inoculation with *F. oxysporum*15 days after *M. enterolobii* inoculation	23.50[a]	15.00[a]	2.95[a]	13.50[a]	14.00[a]	2.60[a]
	(43.33)	(47.55)	(49.46)	(55.16)	(43.79)	(68.29)
*F. oxysporum*followed by inoculation with*M. enterolobii* 15 days after *F. oxysporum*inoculation	33.00[c]	19.00[c]	3.50[b]	16.50[b]	19.00[c]	3.20[a]
	(20.28)	(33.45)	(39.05)	(45.01)	(24.49)	(60.98)
Inoculation of *M. enterolobii*and*F. oxysporum*simultaneously	26.75[b]	14.75[a]	3.90[b]	16.00[b]	16.25[b]	2.80[a]
	(34.00)	(46.68)	(34.06)	(46.59)	(35.72)	(65.85)
Un-inoculated Control	41.50[e]	28.60[e]	5.75	30.00[e]	25.25[e]	8.20[c]
CD (p=0.05)	2.5940	1.7462	0.3633	1.6108	1.5377	0.8391

Figures in parentheses are per cent decrease over control

percent infection was established in the replications of the above treatment. However, in other treatments the wilt establishment rate was lesser. This revealed the complex nature as well as the combined role of both fungus and nematode in the establishment of wilt.

The interaction study revealed that nematode followed by inoculation with fungus 15 days later treatment significantly reduced the growth parameters *viz*., shoot and root length, fresh shoot and root weight and dry shoot and root weight compared to un-inoculated control. Highest per cent reduction in shoot length (43.33), root length (55.16), fresh shoot weight (47.55), shoot dry weight (49.46), fresh root weight (43.79) and root dry weight (68.29) were recorded. Number of females (45.5), egg masses (40), nematode population (332) and gall index (3.25) of *M. enterolobii* were significantly higher in plants inoculated with nematode alone.

Less number of females (35.5), egg masses (30.5) and nematode population (300.75) and gall index (1.5) were recorded in the plants inoculated with fungus followed by nematode. The wilt causing fungus when inoculated prior to nematode adversely affected the nematode multiplication. Maximum number of spores of *F. oxysporum* (CFU 62 x 10^3) was observed in fungus alone treatment and minimum number of spores (CFU 18 x 10^3) was recorded in fungus followed by nematode inoculation. The root knot index (3.25) was significantly higher in plants inoculated with nematode alone followed by nematode 15 days prior to fungus (3.5), nematode and fungus simultaneously (2.75) and fungus 15 days prior to nematode (1.5) and fungus alone inoculated plants (0.0). Disease incidence percentage was highest (50) in fungus alone, nematode followed by fungus 15 days later and

Table 2. Effect of combinations of *M. enterolobii* and *F. oxysporum*on nematode populations and disease incidence

Treatment	*M. enterolobii* parameters				disease incidence (%)	No. of days taken for symptom expression
	J_2/250 g of soil	Female/ 5g root system	Egg mass/ 5g root system	Root knot index		
Nematode alone	332[a] (15.37)	45.5[a] (6.69)	40[a] (6.36)	3.25	0.00	-
Fungus alone	0.00[e] (0.71)	0.00[e] (0.71)	0.00[e] (0.71)	0.00	50.00	60
Nematode followed by inoculation with fungus 15 days later	317[b] (17.82)	39.75[b] (6.34)	35.5[b] (6.00)	3.50	100.00	45
Fungus followed by inoculation with nematode 15 days later	300.75[d] (17.36)	35.5[d] (6.00)	31.5[c] (5.66)	1.50	75.00	55
Inoculation of *M. enterolobii*and *F. oxysporum*simultaneously	306.75[c] (17.53)	35.75[c] (6.02)	30.5[d] (5.57)	2.75	100.00	52
Un-inoculated Control	0.00[e] (0.71)	0.00[e] (0.71)	0.00[e] (0.71)	0.00	0.00	-
CD (p=0.05)	3.53	0.12	0.08	-	-	-

Figures in parentheses are square root transformed values.

simultaneous inoculation of both nematode and fungus (Table 1 and 2).

DISCUSSION

Interaction between *M. enterolobii* and *F. oxysporum*in guava

The present investigation revealed that inoculation of seedlings with either *M. enterolobii* or *F. oxysporum*significantly reduced the growth parameters *viz*., shoot and root length, fresh shoot and root weight and dry shoot and root weight compared with the un-inoculated control. Similar reduction in plant growth parameters were reported by Jonathan and Rajendran (2000) and Patel *et al* (2000) in chickpea.

Number of females, egg masses and nematode population of *M. incognita* was significantly higher in plants inoculated with nematode alone. Less number of females, egg masses and nematode population was recorded in the plants inoculated with fungus followed by nematode. The root rot fungus when inoculated prior to nematode adversely affected the nematode multiplication. This result is in accordance with earlier findings of Fazal*et al.* (1998) for the interaction of *M. javanica* and *Rhizoctonia bataticola* on black-gram.

In the present study, the disease incidence was highest (100 per cent) in *M. enterolobii* followed by inoculation with *F. oxysporum*15 days after *M. enterolobii* inoculation and simultaneous inoculation of the nematode and fungus and nematode followed by fungus. This is in agreement with the findings of *M. incognita* and *F.oxysporum* on *Vigna radiata*. This might be due to formation of nutrient rich cells at the feeding sites of nematodes that forms suitable substrate for fungal colonization (McLean and Lawrence, 1993; Abdel-Momen and Starr, 1998), whereas decrease in the rate of nematode multiplication from combined inoculations showed antagonistic effect of the fungus on the development and reproduction of nematodes. This can be attributed to the possible toxic effect of fungal metabolites in the presence of *F. oxysporum,* or may be

due to destruction of root tissues by the fungus before the completion of nematode life cycle, or because of certain physiological and biochemical changes in the host as a result of nematode and fungus interaction.

However, the plants inoculated with *F. oxysporum* alone recorded minimum root infection than the plants where the nematode was present together with fungus. The minimum root infection in such plants suggests the delay in the entry of fungus due to absence of predisposing agent (*M. incognita*) or due to the absence of nutrient rich cells, which are responsible for attracting the fungus to the galled roots (Golden and Van Gundy, 1975).

The inoculation studies have conclusively established that a disease complex exists between *M. incognita and F. oxysporum*. According to Brodie and Cooper (1964)the percentage of cotton plants killed by *Pythium aphanidermatum* was higher in *M. incognita* infested soil. The results of the present investigation suggest that *M. enterolobii* and *F. oxysporum* together caused greater damage to guava than either of them alone and in order to obtain maximum yield, prophylactic management practices must be taken against both these pathogens.

ACKNOWLEDGMENT

The publication is a part of M.Sc., (Ag) thesis. I would like to thank the Department of Nematology, Centre for Plant Protection Studies and Dean (School of Post Graduate Studies) and Tamil Nadu Agricultural University for their support.

REFERENCES

Abdel-Momen, S. M.and Starr, J. L. 1998. *Meloidogynejavanica-Rhizoctoniasolani* disease complex of peanut. *Fundamental and applied nematology*, **21**(5): 611-616.

Anonymous,2018.Data base. Area and production of horticulture, fruit crops at http://www.nhb.gov.in/nhm.com

Atkinson, G. F. 1892. Some diseases of cotton (No. 41).Agricultural Experiment Station of the Agricultural and Mechanical College.

Brito, J. A., Stanley, J. D., Mendes, M. L., Cetintas, R., and Dickson, D. W. 2007. Host status of selected cultivated plants to *Meloidogynemayaguensis* in Florida. *Nematropica*, **37**(1): 65-72.

Brodie, B. B., & Cooper, W. E. 1964.Relation of parasitic nematodes to postemergence damping-off of cotton. *Phytopathology*, **54** (8): 1023-1027.

Fazal, M., Bhat, M. Y., Imran, M. and Siddiqui, Z. A. 1998.A disease of blackgram involving *Meloidogynejavanica* and *Rhizoctoniabataticola*. *Nematologia Mediterranea*, **26** (1): 63-66.

Golden, J. K., & Van Gundy, S. D. 1975.A Disease Complex of Okra and Tomato Involving the Nematode, *Meloidogyne incognita*, and the Soil-Inhabiting Fungus. *Rhizoctoniasolani Phytopathology*, **65:** 265-273.

Gomes, C. B., Couto, M. E. O., and Carneiro, R. M. D. G. 2008.Occurrence of *Meloidogynemayaguensis* on guava and tabacco in South of Brazil. *Nematologia Brasileira*, **32**(3): 244-247.

Hays, W.B. 1970.Fruit growing in India, Kitabistan, Allahabad, 297.

Jonathan, E. I., and Rajendran, G. 2000.Pathogenic effect of root-knot nematode, *Meloidogyne incognita* on banana, *Musa* sp. *Indian Journal of Nematology*, **30**(1), 13-15.

McLean, K. S., and Lawrence, G. W. 1993. Interrelationship of *Heterodera glycines* and *Fusariumsolani* in sudden death syndrome of soybean. *Journal of Nematology*, **25**(3), 434.

Patel, B. A., Patel, D. J., and Patel, R. G. 2000.Interaction between *Meloidogyne incognita* and wilt inducing fungus, *Fusarium oxysporum* f. sp. *ciceris* on chickpea cv. Dahod Yellow. *Indian Journal of Nematology*, **30**(2): 133-135.

Poornima, K., Suresh, P., Kalaiarasan, P., Subramanian, S. and Ramaraju, K. 2016. Root Knot Nematode, *Meloidogyne enterolobii* in Guava (*Psidium guajava* L.) A New Record from India. *Madras Agricultural Journal*, 103.

Rammah, A and Hirschmann, H.1988. *Meloidogynemayaguensis* n. sp.(Meloidogynidae), a root-knot nematode from Puerto Rico. Journal of *Nematology*, **20**(1): 58.

MS Received 5 November 2019
MS Accepted 10 December 2019