academicJournals

Vol. 9(18), pp. 1281-1285, 6 May, 2015
DOI: 10.5897/AJMR2015.7450
Article Number: D347EFB53186
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Meloidogyne incognita and *Fusarium oxysporum* interaction in Gerbera

K. Sankari Meena[1]*, S. A. Ramyabharathi[2], T. Raguchander[2] and E. I. Jonathan[1]

[1]Department of Nematology, Tamil Nadu Agricultural University, Coimbatore-641 003 Tamil Nadu, India.
[2]Department of Plant Pathology,Tamil Nadu Agricultural University, Coimbatore-641 003 Tamil Nadu, India.

Received 26 February, 2015; Accepted 20 April, 2015

Pot culture experiments were carried out to study the interactive effect of root knot nematode, *Meloidogyne incognita* and the fungus, *Fusarium oxysporum* in Gerbera, *Gerbera jamesonii* Hook. Sequential and concomitant inoculation of the nematode and fungus was done to observe the disease severity in the plants due to the individual effect and interactive effect of nematode and fungus. It was observed from the study that the wilt disease was found to be more severe with the sequential inoculation of nematodes followed by fungus than with the fungus alone treatment. From the study, it was clear that nematodes act as a predisposer in the spread of secondary fungal pathogens.

Key words: Gerbera*, Meloidogyne incognita, Fusarium oxysporum.*

INTRODUCTION

Gerbera (*Gerbera jamesonii* Hook) is a popular cutflower widely used as a decorative garden plant. It is the fifth most used cutflower in the world. In India, there is an increase in demand for this cutflower which is fetching one of the important commercial trades in agriculture. Growing of this cutflower has emerged as a high tech activity under controlled climatic conditions inside the green house. Under cultivation, the crop is affected by several biotic stresses among which the most significant damage is caused by the root knot nematode, *Meloidogyne* species. These nematodes are polyphagous in nature and can be controlled only by integrated management practices. Infection of this nematode results in heavy loss to the crops both in quality and quantity. Infection of this nematode can be easily recognized by the formation of prominent root galls at the infection site. Though, yield loss due to this nematode is difficult to predict, approximate yield loss due to this nematode has been predicted by many authors in various crops. Nagesh and Reddy (2000) estimated the yield loss in gerbera due to *Meloidogyne incognita* infection as 31.1%. Another important biotic stress to which the crop exposed is the fungus, *Fusarium oxysporum* f. sp. *gerberae.* More than 70% of major crop diseases are caused by fungi and cause significant yield loss in most of the horticultural crops (Agrios, 2005). Controlled climatic condition in the green house favours the development of *F. oxysporum* in gerbera and cause severe economic loss to the farmers

*Corresponding author. E-mail: meenaa5@rediffmail.com; sankarimeena.agri@gmail.com. Tel: 08903240897.

(Rajendran et al., 2014), which results in early death of the plant.

Presence of nematodes even breakdown the resistance of the plants to *Fusarium* infection (France and Abawi, 1994). Intensity of the damage caused by the nematode-fungus disease complex is more severe in plants than their separate infection (Jonathan and Gajendran, 1998; Jeffers and Roberts, 2003). Generally, root knot nematode-fungus interaction is considered to be one of the important factors responsible for the crop reduction and very little work has been reported on the nematode-fungus interaction in gerbera. This research was proposed to study the intensity of damage caused due to nematode-fungus disease complex in gerbera.

MATERIALS AND METHODS

Source and identification of root knot nematode associated with gerbera

Inoculum of root knot nematode was obtained from the nematode infected gerbera field at Coimbatore, Tamil Nadu, India. Root knot nematode egg masses were extracted from the roots of the gerbera. For the extraction, galled roots of the plants were collected and washed in water to remove the adhering soil particles and the protruding egg mass in the galls were collected under microscope with the needle. The collected egg masses were placed in distilled water for hatching. After 24 - 48 h, the entire eggs were hatched into juveniles and the freshly hatched juveniles were inoculated into one month old tomato plants cv. Co 3, planted in 5 kg mud pots filled with sterilized pot mixture at one juvenile per g soil (5000 juveniles / pot). After 30 days of inoculation of nematodes into tomato plants, eggs were extracted from tomato roots with the above mentioned procedure and used for the interaction studies.

Meanwhile, root knot nematode females collected from the gerbera roots were processed for perineal pattern to confirm the species of root knot nematode associated with the plant.

Preparation of perineal pattern of root knot nematode

Matured *Meloidogyne* females were teased from the root galls and placed on a glass slide. They were cut at the neck region and body tissues were gently pushed out. The cuticle was placed in 45 per cent lactic acid to facilitate further cleaning. The cuticle was carefully trimmed so that 5-10 times the perineal area (area near to vulva) was retained. This was transferred to a fresh drop of glycerol and examined under microscope.

Source and identification of *Fusarium* species associated with gerbera

Inoculum of *Fusarium* was obtained from the wilt infected gerbera plants collected at Coimbatore, Tamil Nadu, India. For the isolation of *Fusarium* species, a small section of the infected root (5-6 mm) tissues were cut and placed on Potato Dextrose Agar (PDA) medium with an antibacterial agent (streptomycin sulfate). The plate was incubated for 2-4 days. Conidial culture was prepared from the specimen to confirm the species.

Preparation of conidial culture

The sporulated hyphae was scrapped from the PDA medium and placed on a cavity slide. Few drops of water were added to the slide and mixed well with the hyphae. The slide was checked under microscope to observe the conidial characters.

Nematode-fungus interaction in gerbera

Uniform sized, healthy tissue culture plants of gerbera var. Palmbeach obtained from Spic Biotech, Coimbatore, India were planted in 5 kg pots filled with sterilized pot mixture (red soil: sand: Farm Yard Manure (FYM): 2:1:1). One month after planting, the plants were inoculated with the fungus, *F. oxysporum* and the nematode *M. incognita* as mentioned in Table 1 at the rate of 50 ml conidial suspension (Ramamoorthy et al., 2002; Ramyabharathi and Raguchander, 2014) per pot and 5000 second stage juveniles of nematode per pot. Microconidial suspension of the fungi was prepared by pouring 20 ml of sterile distilled water in each Petri plate. Concentration of microconidia was adjusted to 1000 conidia/ml. Nematode and fungus inoculation was made by carefully adding the homogenous suspension of the two pathogens at the root zone of the plants.

Experiments were laid out in completely randomized design with four replications during the months of April to May, 2013 and repeated during the months of July to August, 2013; October to November, 2013 and December 2013 to January 2014 in the greenhouse at Tamil Nadu Agricultural University, Coimbatore, India maintaining the temperature range of 24 - 32°C.

Evaluations were performed 45 days after inoculation. Measurements were made on the plant growth parameters (shoot length and weight; root length and weight) and yield parameters: number of flowers per plant; flower diameter (cm) and stalk length (cm). Observations were made on the root population of nematode *viz*., number of females per g root, number of egg mass per g root and gall index of 1-5 scale (Gall index: 1=no galls; 2=1-25% galls; 3=26-50% galls; 4=51-75% galls; 5=76 -100% galls per root system) (Taylor and Sasser, 1978). Nematode population in soil was processed as per the sieving method of Cobb and Modified Baermann funnel technique. Per cent wilt incidence due to fungus was assessed using number of wilt infected plants /total number of plants taken for observation.

Data were analysed using analysis of variance (ANOVA). In the experiments that were repeated, since the error variances were similar, the analysis was performed on pooled data. Treatment means were compared and critical differences (CD) was calculated at P=0.05 to test for significant differences between treatments (T) (Panse and Sukhatme, 1978).

RESULTS

Identification of *Meloidogyne* species associated with gerbera

Meloidogyne species collected from gerbera were identified by the cuticular markings in the perenial area of the matured female. All the root knot nematode species observed were with high dorsal arch which were flattened at the top (Plate 1). This confirmed the species as *M. incognita.*

Identification of *Fusarium* species associated with gerbera

The species was identified based on the morphological characters. Observation under microscope revealed small, oval shaped, single or bicelled microconidia and

Table 1. Interactive effect of *Meloidogyne incognita* and *Fusarium oxysporum* in Gerbera.

Treatments	Plant growth parameters							Nematode population				
	Shoot		Root		Stalk length (cm)	Flower diameter (cm)	No. of flowers/plant	Root population			Soil population	Per cent wilt incidence
	length (cm)	weight (g)	length (cm)	weight (g)				No. of females/g root	No. of egg mass/ g root	Gall index (0-5 scale)	No. of juveniles/ 250 cc soil	
Nematode alone	31.85	35.70	19.93	31.88	37.88	8.20	2.00	46.75 (43.17)	22.75 (28.42)	5 (12.92)	347.5 (18.95)	6.5(2.54)
Fungus alone	31.60	38.13	20.78	41.08	37.95	8.70	1.75	0 (4.05)	0 (4.05)	0 (4.05)	0(4.05)	74.5 (8.62)
Concomitant inoculation of nematode and fungus	24.75	17.78	14.18	11.88	29.10	7.38	1.50	34.5 (35.95)	20.75 (27.07)	3.5 (10.75)	283.75 (16.88)	83.25 (9.11)
Nematodes 15 days prior to fungus inoculation	22.23	9.08	12.35	8.35	27.38	6.45	1.25	42.25 (40.53)	24 (29.30)	4 (11.48)	320.5 (17.53)	95.5 (9.76)
Fungus 15 days prior to nematode inoculation	28.65	23.20	18.28	28.63	32.45	7.43	1.75	20.5 (26.87)	10.75 (19.05)	3 (9.97)	249.75 (16.28)	74.25 (8.61)
Uninoculated control	35.50	43.47	22.90	46.81	46.93	9.35	3.50	0 (4.05)	0 (4.05)	0 (4.05)	0 (4.05)	0 (0.70)
SEd	1.04	0.88	0.82	0.57	0.50	0.24	0.48	1.91	1.57	0.29	2.79	1.18
CD (0.05)	2.19	1.84	1.72	1.20	1.05	0.51	1.01	4.02	3.29	0.61	5.85	2.49

*Pooled data of four pot culture experiments carried out in the green house. Figures in the parentheses in nematode population analysis are square root of X+0.5 transformed values; figures in parentheses for wilt incidence are arcsine transformed values. Gall index: 1=no galls; 2=1-25 % galls; 3=26-50% galls; 4=51-75 % galls; 5=76 -100 % galls per root system. SEd: Standard error difference; CD: Critical Differences.

hyaline, multicelled macroconidia with 3 septation which were sickle shaped with knotched base at one end. This confirmed the species as *Fusarium oxysporum* (Plate 2).

Effect of individual inoculation of *M. incognita* and *F. oxysporum* on galls and wilt incidence

Nematode and fungus, when inoculated individually caused significant reduction in the plant growth parameters of gerbera when compared to untreated control (Table 1). Highest gall index of 5.0 and wilt incidence of 6.5 per cent was observed with the inoculation of nematodes alone to the pots. Fungus inoculation into the plants showed no galls in their roots, with however, 74 per cent wilt prevalence.

Effect of *F. oxysporum* and *M. incognita* on *Fusarium* wilt incidence

Initial symptoms of chlorosis and wilt incidence were observed as early as 7 days after inoculation of the fungus. In the sequential and concomitant inoculation of nematodes and fungus, wilt incidence was higher than with the fungus alone. Presence of nematode contributed to the early onset of wilt symptom which resulted in more stunting of plants. Sequential inoculation of nematodes 15 days prior to fungus significantly increased the severity of wilt incidence to 95.5 per cent followed by the concomitant inoculation of nematode and fungus which showed 83.25 per cent of wilt incidence indicating that nematodes predispose plants to infection by fungus and aggravate the disease incidence which ultimately leads to plants death.

Effect of *F. oxysporum* and *M. incognita* on the severity of root galls

Concomitant and sequential nematode and fungus inoculation resulted in a significant reduction of the gall index. Gall index of 4.0 was observed when the nematodes were inoculated 15 days prior to the fungus. The index was reduced with concomitant and sequential inoculation of fungus 15 days prior to nematodes. Severity of root galling diminished as the *Fusarium* density increased due to rotting of roots. This reduced the reproduction potential of the nematode. Similarly,

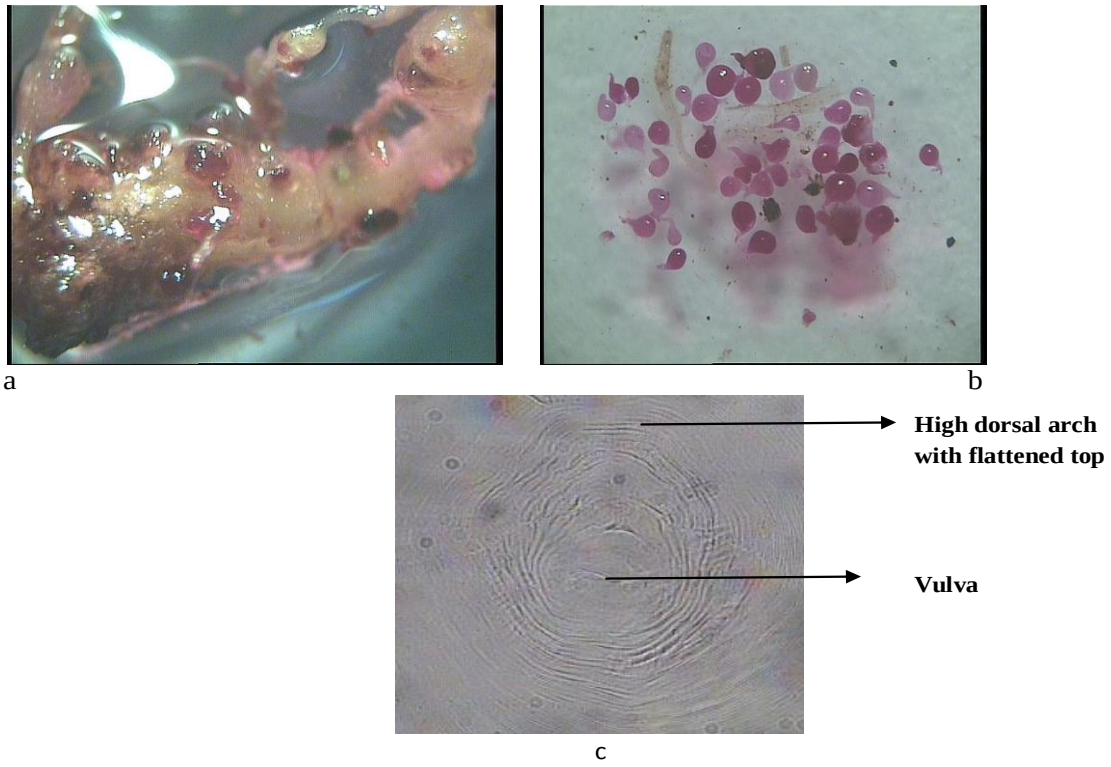

Plate 1. **a.** Root galls of gerbera; **b.** Root knot nematode females collected per g of gerbera root; **c.** Perennial pattern of root knot nematode, *M. incognita.*

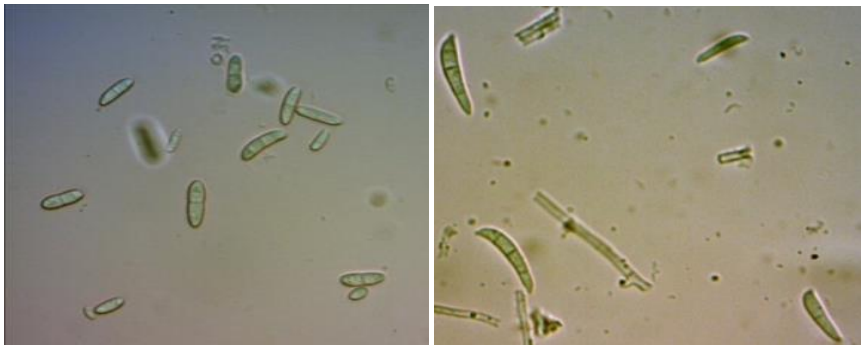

Plate 2. Conidial structures of *Fusarium oxysporum.*

lower nematode female density in roots and juvenile population in the soil were observed in the sequential inoculation of fungus 15 days prior to nematodes followed by the concomitant inoculation of nematodes and fungus.

DISCUSSION

Interaction between nematodes and fungus have been reported previously in various crops *viz.,* muskmelon (Bergeson, 1975), tomato (Sidhu and Webster, 1977), *Vigna unguiculata* (Harris and Ferris, 1991), betelvine (Jonathan et al., 1996) and banana (Jonathan and Gajendran, 1998). Studies observed from the interaction in various crops revealed that nematodes predisposed the plants to the secondary infection by the fungal pathogens which were in agreement with the present study. Concomitant and sequential inoculation of nematodes and fungus resulted in the aggravation of wilt disease severity in gerbera compared with their individual inoculation. This was in accord with the study conducted by Jonathan and Gajendran (1998) in banana cv. Rasthali, where they observed that the incidence of

panama wilt disease was severe with the sequential inoculation of *M. incognita* followed by the fungus, *F. oxysporum* f. sp. *cubense* and concomitant inoculation of the two pathogens. Breakdown of resistance to *Fusarium* by prior infection with *Meloidogyne* has been reported in chickpea (Sharma et al., 1992), cotton, cucumber (Fritzsche et al., 1983), soybean, tomato (Bowman and Bloom, 1966; Sidhu and Webster, 1977). Severe wilt incidence due to fungus was observed in tobacco plants pre inoculated with nematodes (Porter and Powell, 1967). Nematode activities in roots modify roots physiology and morphology (Sankari Meena et al., 2011) which make the plant more vulnerable to the infection by secondary pathogens (Mayol and Bergeson, 1970). El-Shawadfy et al. (1988) suggested that presence of fungus did not altered the invasion of nematodes into the roots but affected the development of nematode females and severity of galls which might be due to the invasion of giant cells of nematodes by the *Fusarium*. This ultimately restricts the nematode reproductive potential in the plant. Moreover, toxic metabolites produced by the fungi may also reduce the egg hatching of nematodes and immobilize the second-stage juveniles of them (Fattah and Webster, 1989). These findings were confirmed by the present study where reduced nematode population and gall index with increased wilt incidence were observed with concomitant nematode and fungus inoculation followed by sequential pathogens inoculation. Thus it was proved from the present study that presence of nematode paves way for the early entry of the fungus into the plants which aggravate the wilt disease severity than the individual inoculation of nematodes and fungus.

Conflict of interests

The author(s) did not declare any conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the Department of Biotechnology, Ministry of Science and Technology for providing the financial assistance to carry out the research work.

REFERENCES

Agrios GN (2005). Plant Pathology. Fifth edition, Academic Press. New York, p. 633.

Bergeson GB (1975). The effect of *Meloidogyne incognita* on the resistance of four muskmelon varieties to *Fusarium* wilt. Plant Dis. Rep. 59:410-413.

Bowman P, Bloom JR (1966). Breaking the resistance of tomato varieties to *Fusarium* wilt by *Meloidogyne incognita.* Phytopathology 56:871.

El-Shawadfy, Moussa M, Nigel G, Hague M (1988). Influence of *Fusarium oxysporum* f. sp. *glycines* on the invasion and development of *Meloidogyne incognita* on soybean. Revue de Nematol. 4:437-439.

Fattah FA, Webster JM (1989). Ultrastructural modifications of *Meloidogyne javanica* induced giant cells caused by fungal culture filtrates. Revue de Nematol. 12: 197 - 210.

France RA, Abawi GS (1994). Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp*. phaseoli* on selected bean genotypes**.** J. Nematol. 26: 467-474.

Fritzsche R, Pelcz J, Oettel G, Thiele S (1983). Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum* in greenhouse cucumbers. Tagungsbericht. 216: 685 - 690.

Harris AR, Ferris H (1991). Interactions between *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne* spp. in *Vigna unguiculata.* Pathogenesis by *F. o. tracheiphilium* as affected by *M. javanica* and host cultivar. Plant Pathol. 40: 465-475.

Jeffers DP, Roberts PA (2003). Effect of plant date and host genotype on the root-knot-*Fusarium* wilt disease complex in cotton. Phytopathology 83: 645 - 654.

Jonathan EI, Gajendran G (1998). Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *cubense* on banana. Nematol. Medit. 26: 9-11.

Jonathan EI, Sivakumar M, Padmanabhan D (1996). Interaction of *Meloidogyne incognita* and *Phytophthora palmivora* on betelvine. Nematol. Medit. 24: 341 - 343.

Mayol PS, Bergeson GB (1970). The role of secondary invaders in *Meloidogyne incognita* infection. J. Nematol. 2: 80 - 83.

Nagesh M, Parvatha Reddy P (2000). Crop loss estimation in carnation and gerbera due to the root-knot nematode, *Meloidogyne incognita* (Kofoid etWhite) Chitwood. Pest Mange. Hortic. Ecosyst. 6: 158-159.

Panse VG, Sukhatme PV (1978). Statistical Methods for Agricultural Workers. Edn 2.197 pp. Indian Council of Agricultural Research, New Delhi.

Porter DM, Powell NT (1967). Influence of certain *Meloidogyne* species on *Fusarium* wilt development in flue-cured tobacco. Phytopathology 57:282-285.

Rajendran L, Raja P, Jegadeeswari V, Santhi VP, Selvaraj N (2014). *Pseudomonas fluorescens* and *Trichoderma viride* enriched bioconsortium for the management of *Fusarium* wilt in carnation and gerbera under protected cultivation. Indian Phytopathol. 67(1): 77-81.

Ramamoorthy V, Raguchander T, Samiyappan R (2002). Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici.* Plant Soil 239: 55 - 68.

Ramyabharathi SA, Raguchander T (2014). Induction of defense enzymes in response to treatment with *Bacillus subtilis* EPCO 16 liquid formulation. J. Mycol. Plant Pathol. 44(1):122-125.

Sankari Meena K, Jonathan EI, Kavitha PG (2011). Efficacy of *Pseudomonas fluorescens* on the histopathological changes in tomato roots infested with root knot nematode, *Meloidogyne incognita.* Madras Agric. J. 97(10-12): 96-398.

Sharma SB, Smith DH, Mc Donald D (1992). Nematode constraints of chickpea and pigeonpea production in the semiarid tropics. Plant Dis. 76:868-874.

Sidhu G, Webster JM (1977). Predisposition of tomato to the wilt fungus *(Fusarium oxysporum lycopersici)* by the root-knot nematode *(Meloidogyne incognita).* Nematologica 23:436-442.

Taylor AL, Sasser JN (1978). Biology, identification and control of root knot nematode (*Meloidogyne* spp.). Coop. Pub. Dep. Plant Pathol., North Carolina State Univ. and U.S. Agency Int. Dev. Raleigh, N.C., 111.