I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS) Rating : 3.03

Incidence and Prevalence of Gerbera Fusarium wilt in Association with Nematodes in Tamil Nadu

SA. Ramyabharathi[*#], K. Sankari Meena[**], T. Raguchander[*] and E. I. Jonathan[**]

ABSTRACT: *Survey was carried out in Coimbatore, Nilgiris, Dindigul, Salem and Krishnagiri districts of Tamil Nadu during 2012-13 to find out the incidence and severity of wilt disease complex of Gerbera jamesonii Bolus ex Hooker f. Out of 26 gerbera fields surveyed maximum per cent wilt incidence (98.43%) was observed in Maruthur, Coimbatore district, Tamil Nadu with the root-knot nematode (297.00) and Helicotylenchus (85.33) population per 250g of soil. Wilt infected roots were collected, cultured on Fusarium selective medium and confirmed as Fusarium oxysporum f. sp. gerberae based on the morphological identification and pathogenicity. The survey resulted in maximum per cent wilt incidence due to Fusarium oxysporum and root knot nematode complex in gerbera growing areas which adversely affected the growth, quality and yield of cut flowers.*

Keywords: *Gerbera cultivars, Wilt survey, Fusarium oxysporum, Meloidogyne incognita*

INTRODUCTION

Gerbera are widely used as cut flowers because the flowers are available in numerous colours like pink, white, yellow, orange, crimson, purple and in many combinations and shades. The flowers are well suited for vase decoration and other floral arrangements on account of its lengthy robust pedicel. Gerbera occupies seventh position in top ten cut flowers of export (Parthasarathy and Nagaraju, 1999). Good quality cut flowers of gerbera can be produced under protected cultivation. India being gifted with best climate for protected cultivation, the production of gerberas, particularly during winter months is highly profitable when compared to temperate countries, where these are grown under green house conditions. The crop is subjected to attack by many diseases *viz*., stem blight, leaf spot, bacterial root rot, dry root rot, aerial blight etc. Among these, root-knot and wilt disease complex is occurring in severe form and cause heavy losses. Plant parasitic nematodes are found in all agricultural regions of the world and any crop is likely to suffer from these parasites. They may also be additive to other combination of plant parasitic nematodes with fungal pathogens, which is sufficient to induce heavy crop losses (Zacheo Giuseppe, 1993). Predisposition of fungal diseases by plant parasitic nematodes requires a minimum level of nematode infestation (Garber *et al.* 1979). This survey, aimed at assessing the incidence and severity of the diseases in gerbera fields in and around TamilNadu. Also, to isolate and identify the pathogenic species of fungi and nematodes associated with the wilt disease of gerbera in the surveyed areas.

MATERIALS AND METHODS

The research was carried out in Department of Plant Pathology, TamilNadu agricultural university, Coimbatore, India during the year 2013.

Soil survey and sampling: Five different geographical locations of Tamil Nadu were surveyed and samples of plants along with the soil were collected from gerbera fields. Collection of plant and soil samples was done from twenty six fields i.e, Coimbatore (Maruthur 2 fields; Karamadai 2 fields; Madukarai 2 fields), Nilgiris (pudumanthu 4 fields; old ooty 2 fields; Conoor 4 fields), Dindigul (Thadiyankudisai 1 field; Retiyarsathiram 1 field; Kodaikanal 1 field), Salem (Yercaud HRS 1 field;

* Department of Plant Pathology

** Department of Agricultural Nematology, Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

#Corresponding Author, *E-mail: ramyabharu@gmail.com*

Manjakuttai 1 field; Asampur 1 field) and Krishnagiri (Denkanikottai 1 field; Sandhanapalli 3 fields) (Table 1). Samples of soil and roots were collected from infected fields from the rhizosphere of gerbera crop to the root depth, in the similar manner totally about 10-15 spots were selected randomly for taking soil and root samples representing the whole field. Later from this, composite samples of 250 g of soil and 5 g of root were formed. Randomly 100 plants were selected in different locations in a field and numbers of plants wilted were counted and the mean wilt incidence was expressed in percentage. Whenever required, the complete wilted plants are also collected. The per cent disease incidence was calculated by using the following formula.

Per cent disease incidence = (Number of plants affected / Total number of plants observed) × 100

Each sample was filled in polythene bag and tied with a rubber band labeled immediately. Information pertaining to the locality, crop history, *etc.* was also obtained along with the samples. Samples of soil and roots were analyzed on the day of collection or after keeping for a few days under refrigerator condition.

The nematode populations from soil and root samples were estimated. The root samples were also used for detection of the fungus associated with wilted plants.

Estimation of Nematode Population in Soil

Cobb's sieving and decanting technique was followed for which 250g of the soil samples was taken in a container and mixed thoroughly with water. Hard particles and stones were removed by stirring the suspension and were then passed through a set of sieves of 250, 45 and 37 μm pore size.

The sievates were collected on a tissue paper spread over a coarse mesh, which was then placed in a petridish containing enough water to keep the tissue paper (placed on coarse mesh) always moist. This assembly was kept still for three days, care was taken to prevent drying of the tissue paper. The nematode suspension collected in the Petri dish was examined by means of research stereobinocular microscope. The different plant parasitic nematodes present in the suspension were identified. Their number present in the suspension were determined by taking the average number of nematodes present in five different one milliliter aliquots of nematode suspension.

Estimation of Nematode Population from Root Samples

Root samples of known quantity (1 gram) were directly observed under stereobinocular microscope for counting adult females of sedentary nematodes

Table 1
Per cent wilt Incidence and Population of Root knot Nematode Associated with Gerbera in Different Districts of Tamil Nadu

			Root knot nematode			
			Root population			
Place	*Variety*	*Age of the crop (months)*	*Female population / g root*	*Egg mass / g root*	*Gall index*	*Soil population / 250 g soil*
Coimbatore district						
Maruthur	Advance	12	12.67	11.67	3.67	297.00
Karamadai	Sunway	12	11.33	9.67	3.33	293.33
Madukarai	Rosalin	8	11.33	9.33	3.33	282.33
Nilgiris district						
Pudhumanthu	Advance	12	8.67	8.00	2.33	203.00
Old ooty	Danaellen	12	12.00	11.33	3.00	246.67
Coonoor	Shimmer	8	13.33	12.67	2.67	222.00
Dindugul district						
Thadiyankudisai	Sunway	12	10.67	10.00	3.33	282.33
Retiyarsathiram	Shimmer	12	11.00	9.00	3.67	293.67
Kodaikanal	Sunway	12	12.33	8.67	4.00	287.67
Salem district						
Yercaud HRS	Shimmer	11	10.67	9.33	3.33	285.00
Manjakuttai	Sunway	11	12.33	8.33	3.67	299.33
Asampur	Rosalin	11	14.00	7.00	4.00	296.67
Krishnagiri district						
Denkanikottai	Sunway	9	9.67	9.00	3.33	213.00
Sandhanapalli - 1	Rosalin	12	11.00	10.33	4.00	276.67
Sandhanapalli - 2	Sunway	12	11.33	13.67	3.67	242.00

and same was processed using blending and Baermann's funnel method for the extraction of active forms of sedentary as well as migratory nematodes. After incubation of 48 hours, the volume of suspension was made to 250 ml out of which 10 ml pipetted out and used for counting nematode. Nematode count from this was finally converted to gram of root. To observe presence of second stage larvae and other developing stages. The roots were stained in a boiling solution of 0.05 per cent lactophenol cotton blue for one minute and allowed to cool for few minutes before washing gently under running tap water. Stained roots were then kept in plain lactophenol for few to 48 hours for differentiation and examined under stereobinacular microscope. The stained nematodes were counted and converted to 1 g root. The total nematode counts were expressed per gram of root. The nematodes were identified based on key provided by Taylor and Sasser (1978).

Counting Egg Masses

The number of egg masses of root-knot nematode per root system was counted by exposing the infected roots to 0.25 per cent trypan blue for three minutes as per the procedure given by Sharma and Ashok Kumar (1991).

Perineal Pattern Preparation and Identification

Perineal patterns were prepared from adult females collected from diseased plants in individual surveyed fields from which nematode species were identified (Taylor *et al*., 1955). At least five slides, each bearing four perineal patterns, were prepared for each surveyed field. Perineal patterns were identified under a compound microscope with the aid of a pictorial key (Eisenback *et al.,* 1981).

Isolation of the fungi: *Fusarium* spp. was isolated from infected root sample of gerbera plants collected from the fields. For fungal isolation from plant, the roots were washed under tap water, chopped into 2 cm small pieces and surface sterilized in 0.5% NaOCl for two minutes then rinsed twice with triple distilled water and placed on Fusarium selective medium (Glycerine 10g, urea 1g,

L-alanine 0.5g, PCNB 1g, Rose Bengal 0.05g streptomycin 0.1g agar 15g in 1litre of distilled water) and finally kept in an incubator at 27°C under dark conditions. All the procedure was carried out in laminar hood under sterilize condition. After five days of incubation, small colonies of fungus appeared, which were picked with a sterilized inoculation needle and transferred to fresh PDA plates.

Pathogenicity

The fungi *viz*., *F. oxysporum* f.sp. *gerberae* was mass multiplied separately in sand maize medium (9:1). One-month-old gerbera seedlings were maintained in earthenware pot (30 cm dia) and were inoculated with the pathogen at the rate of 5g of inoculum per kg of soil.

Morphological characterization of the isolated fungi: *Fusarium* was identified on the basis of colony morphology, morphological characteristic of macro and micro-conidia and chlamydospores. Among these *Fusarium* isolates, *F. oxysporum* species was identified using manual of Booth (1971).

RESULTS AND DISCUSSION

Incidence of Wilt Disease in Surveyed Areas

A survey was carried out in Coimbatore, Nilgiris, Dindigul, Salem and Krishnagiri districts of Tamil Nadu during 2012-013 to find out the incidence (Fig. 1) and severity of the wilt disease complex of *Gerbera jamesonii*. Maruthur village in Coimbatore district recorded the highest percent incidence of 98.43, followed by Yercaud in Salem district with incidence of 40.33 per cent. The least disease incidence of 2.33 per cent was recorded at Denkanikottai in Krishnagiri district of Tamil Nadu. The wilt incidence in the surveyed areas presented in Table 1 and Fig. 2**.**

Figure 1: Places surveyed for the occurrence of root-knot and wilt disease complex in Tamil Nadu

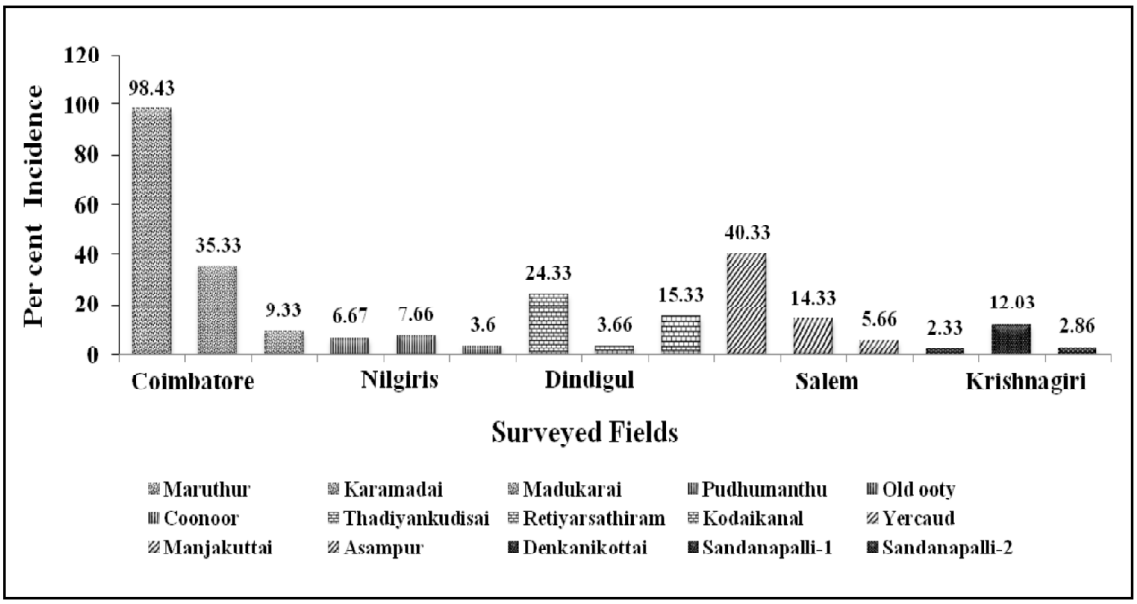


Figure 2: Incidence of wilt disease in different districts of Tamil Nadu

Identification of Wilt Infected Gerbera Plants in Surveyed Areas

Affected plants were stunted and developed yellow leaves with initially brown and eventually black streaks in the vascular system. The vascular streaks in the yellow leaves were continuous with a brown discoloration in the vascular system of the crown and upper taproot (Fig. 3)

Figure 3: Healthy and wilt infected gerbera plants under field condition

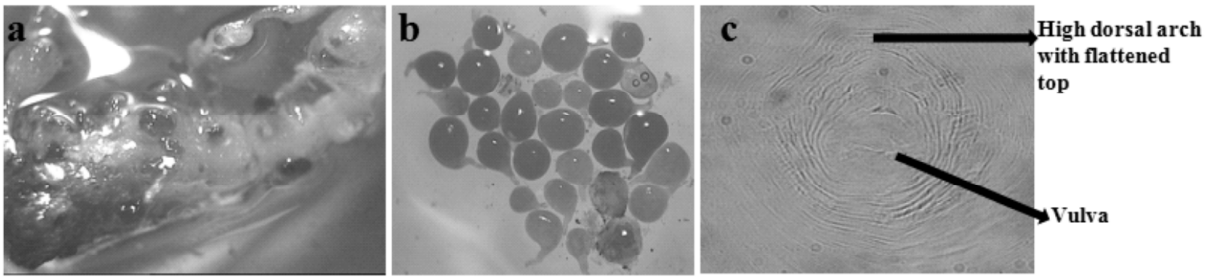
Identification of Nematodes in Surveyed Areas

Root knot nematode population was assessed in both infected root and soil sample (Table 1). In infected soil sample the root knot nematode population was recorded highest (299.33/ 250g of soil) in Manjakuttai followed by Maruthur (297.00/ 250g of soil) and lowest root-knot nematode population per 250g of soil was recorded in Sandhanapalli-2 (242.00). In root sample, root knot nematode egg mass was maximum (13.67/g of root) noticed in Sandhanapalli Krishnagiri district and Asampur, Salem district recorded (7.00/ g of root) the minimum egg mass population. Maximum root-knot nematode female population in infected roots (Fig. 4a, 4b) was recorded in Asampur, Salem district (14.00 / g of root) and lowest was recorded in Pudhumanthu (8.67 g of root). For morphological studies, perineal pattern is the most important morphological characters used for reliable species identification. Using the perineal pattern preparations of the root-knot nematode infesting gerbera grown in Tamil Nadu, the prevailing *Meloidogyne* species was identified as

M. incognita (Fig. 4c). Important diagnostic characters observed were the presence of high, squarish dorsal arch that often continued a distinct whorl in the tail terminal area. The straie were smooth to wavy or zig-zaged and distinct lateral lines. These findings are in confirmation with those mentioned by Eisenback *et al.* (1981) for the root-knot nematode *Meloidogyne incognita.*

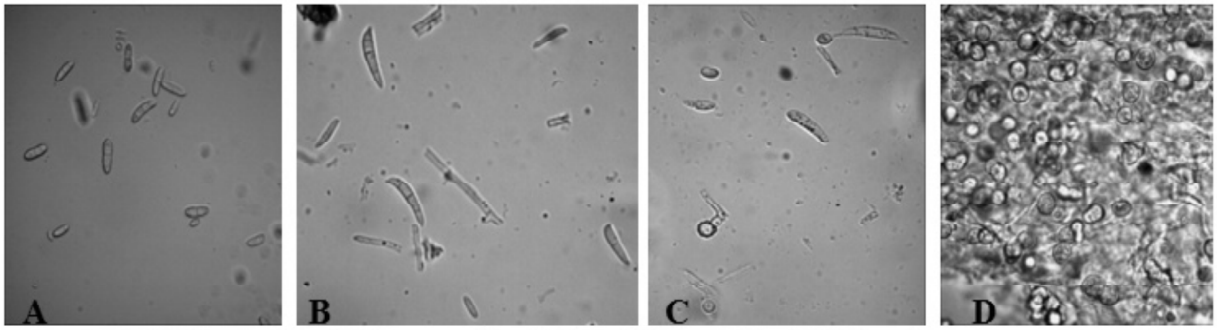
Fungus Associated with Wilt Disease of Gerbera in Surveyed Areas

In all the districts surveyed, it was found that the root wilt caused was *Fusarium oxysporum* when cultured by the standard tissue isolation method on petri plates containing Fusarium selective medium. and produced a dense white mycelial growth. Kaewruang *et al.*, 1989 were also isolated *Fusarium oxysporum* associated with wilt of gerbera. The pathogen *Fusarium oxysporum* was identified microscopically by micro conidia, macro conidia and chlamydospores (Fig. 5). Macroconidia



a. Root knot nematode females in the root galls; b. Root knot nematode females /g root ; c. Perineal pattern of root knot nematodes

Figure 4: Microscopic observation of Root knot nematode *Meloidogyne incognita*



A. Micro conidia; B. Macro conidia; C. Germ tube formation; D. Chlamydospores

Figure 5: Conidia of *Fusarium oxysporum* under light microscope

was sickle shaped with knotched base at one end, hyaline and multicelled with 3 septation. Germ tube formation from macro conidia also observed. Microconidia are small, oval shaped, single or bicelled. The micro conidia number was more when compared to macro conidia. Chlamydospores are one or two celled, thick-walled round spores produced within or terminally on older mycelium or in macroconidia.

The combinations of nematodes and fungus often results in synergistic interaction wherein, the crop loss is greater than expected from either of the pathogens alone or an additive effect of the two together (Francl and Wheeler, 1993). Out of several nematodes of economic importance, root-knot nematodes are the most widely studied and are commonly found involved in synergistic interactions with wilt inducing fungi. From the survey the association of a single fungus *Fusarium oxysporum* with *Meloidogyne incognita* was observed from soil and root samples collected. Similar observations were also made in survey conducted by Krishna Rao and Krishnappa (1994) on chickpea in Karnataka. The findings have further shown that *Fusarium* wilt and root-knot diseases are prevalent in all the study areas. This could be attributed to several factors. It is possible by planting infected tubers, seedlings and continuous cropping of gerbera to meet all-year-round demand may have provided a favourable environment for the growth and development of these organisms. Also, the giant cells induced by the root-knot nematodes might have increased susceptibility to the wilt pathogen.

ACKNOWLEDGEMENT

The authors would like to acknowledge Department of Biotechnology (DBT project No. BT/PR15366/AGR/05/538/2011) India for their financial support.

REFERENCES

Booth, C. (1971), The genus Fusarium. Commonwealth Mycological Institute. London: Kew Eastern Press.

Eisenback, J. D., Hirschmann, H., Sasser, T. N. and Triantaphyllou, A. C. (1981), A Guide to the Four Most Common Species of Root-Knot Nematodes (*Meloidogyne* spp.) with a Pictorial Key. A co-operative publication of the Department of Plant Pathology and Genetics, North Carolina State University and United States Agency for International Development, Releigh, North Carolina.

Francl, L. J. and Wheeler, T. A. (1993), Interaction of plant parasitic nematodes with wilt inducing fungi. In : *Nematode Interactions* (Ed. M. W. Khan), Chapman and Hall, London. 79-103.

Garber, R. H., Jorgensm, E. C., Smith, S. N. and Hyer, A. H. (1979), Interaction of population levels of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on cotton. *J. Nematol.,* **11**: 133-137.

Kaewruang, W., Sivasithamparam, K and Hardy, G.E. (1989), Use of soil solarization to control root rots in gerberas (*Gerbera jamesonii*). *Biol. Fert. soils*., **8**: 38-47.

Krishna Rao, V. and Krishnappa, K. (1994), Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *ciceri* at different inoculum levels on chickpea. *Indian J. Nematol*., **24**: 112-115.

Parthasarathy, V.A. and Nagaraju, V. (1999), *In vitro* propagation in *Gerbera jamesonii* Bolus. *Indian J. Hort.*, **56**: 82–85**.**

Sharma, S. B. and Ashok Kumar, P. (1991), A screening technique to evaluate pigeonpea for resistance to *Rotylenchulus reniformis*. *Ann. Appl. Biol.,* **119**: 323-330.

Taylor, A. L. and Sasser, J. N. (1978), Biology, Identification and Control of Root- Knot Nematodes (*Meloidogyne* spp.) North Carolina State University.

Taylor, A. L., Dropkin, V. H. and Martin, G C. (1955), Perineal pattern of root-knot nematodes. *Phytopathol.,* **45**: 26-34.

Zacheo Giuseppe, (1993), Introduction. In: *Nematode Interaction* (Ed. M. W. Khan), Chapman and Hall, London.