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Exploring the disease severity by the interaction of *Fusarium* wilt and root knot nematode in tomato

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Abstract
Fusarium wilt disease causes economic losses in all tomato growing areas, then it was converted to most devastating disease, when it combined infection with root knot nematode. Wilt infected samples were collected from tomato growing Madukarai area of Coimbatore district. *Fusarium* wilt pathogen was identified based on the morphological and molecular level. Root knot nematode was identified through post cuticular pattern. Then the interaction study was carried out in the pot culture under glass house condition. The present investigation focused on the role of root knot nematode and *Fusarium* pathogen in wilt incidence severity of tomato. The nematode fungal interaction was one of the contributing factor for increasing the wilt incidence and it was confirmed by the prior inoculation of root knot nematode (*M. incognita*) to the fungus (*Fusarium oxysporum* f. sp. *lycopersici*), the wilt incidence was recorded up to 87.50 % and vascular infection was high 53.20 % and 168 galls gram^{-1} of roots were also recorded then followed by 54.50 % wilt incidence, 44.60 % vascular infection and 111 galls gram^{-1} of roots were recorded in simultaneous application of fungus and nematode. The interesting fact is the 175 galls gram^{-1} of root was high in inoculation of nematode alone. The lowest wilt incidence 50.00 % was recorded and 34.90 % vascular infection in the prior inoculation of fungus to the nematode.

Keywords: *fusarium oxysporum* f. sp. *lycopersici*, root knot nematode, interaction, vascular infection

Introduction
Tomato (*Solanum lycopersicum* L.), is one of the most popular vegetable crop grown in the world, next to potato. It is also known as "poor man's apple" belonging to the family Solanaceae. It is native of Peruvian and Mexican region (South America) and is very important in human nutrition (Abubakar, 1999) [1]. Tomato is affected by many diseases by various organisms like fungi, bacteria, virus, viroids and nematodes. Wilting of tomato is the major problem in most of the tomato growing areas. Mostly the wilt disease primarily spreads through contaminated soil, irrigation, diseases saplings and farm implements (Narayanan *et al*., 2015) [11]. In India, *Fusarium oxysporum* is distributed in cosmopolitan nature and it is an anamorphic species that includes numerous plant pathogenic strains causing wilt diseases of a broad range of host plants (Ashwathi *et al*., 2017) [2]. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans, is the most common wilt disease in most of the tomato growing areas. Kawamura and Hirano (1968) [6] reported that the combination of root-knot nematode (*M. incognita)* and wilt fungus (*F. o.* f. sp. *lycopersici*) caused most severe damage to the tomato plants. Objective of the present study deals with the interaction of root knot nematode and *Fusarium* wilt pathogen in wilt disease severity.

Materials and Methods
Isolation and identification of pathogen
Fusarium wilt infected samples were collected from Madukarai area of Coimbatore district. Roots were thoroughly washed, surface sterilized in solution of 0.1 % mercuric chloride for 30 secs, rinsed in five change of sterilized distilled water (SDW), dried on a tissue paper and then incubated on potato dextrose agar (PDA) media for 4 days at room temperature (28±2°C). Hyphal tips are plugged from fungal colonies growing out of stem tissue and infected roots were transferred to sterilize PDA medium and incubated at room temperature. The culture of the isolate was purified by subculturing from the growth of different colonies. From the purified cultures the isolate was stored on slant culture tube at 4°C as stock culture for further studies and future purpose.

Fusarium Wilt – Vascular Discoloration

Preparations of *Fusarium* wilt pathogen inoculum

Fine sand and ground maize seeds were mixed at the ratio of 9:1 respectively with fifty % moisture content, filled in polypropylene bags at 3/4th level and steam sterilized at 121°C at 15 psi for 2 h for two consecutive days. After sterilization, separately 9 mm mycelial disc of *F. o.* f. sp. *lycopersici* (10 isolates) was inoculated in sand maize medium and incubated for 15 days at room temperature (28±2˚C) for multiplication (Riker and Riker, 1936) [12].

Collection of juveniles of root knot nematode

Root knot nematode infected roots of tomato plants were collected from madukarai area of Coimbatore district. Soil was carefully removed from the root portion by washing gently under running tap water. Egg masses were picked from young root knot galls and kept under room temperature (28±2˚C) for hatching in 50ml water in a 100ml beaker. After 48-72 h juveniles hatched were used to inoculate 14 days old tomato seedlings grown in sterilized pot mixture soil (red soil: sand: farm yard manure @ 1:1:1 w/w/w) in 15 cm diameter pots. Pot mixture was sterilized in autoclave at 121˚C at 15 psi for 2 h for two consecutive days and maintained in glasshouse.

Morphological identification of root knot nematode

The roots infested with root knot nematode were washed. The females were dissected out from well-developed galls of the roots under zoom stereo binocular microscope and transferred to petri plate containing water. The posterior portion of the female was cut with scapel (Taylor and Sasser, 1978) [16] and the body contents were cleared. The cleaned posterior portion of female was further trimmed and transferred to a drop of glycerin on a clean glass microscopic slide to observe post cuticular pattern. Glass wools were placed around the portion to avoid damage to it. A cover slip was placed and sealed with nail polish and observed under compound microscope. The species confirmation was based on the perineal pattern described by Chitwood (1949) [3].

Hatching of juveniles and inoculation

The egg masses from stock culture were transferred carefully to a wire gauge sieve containing two layers of facial tissue paper trimmed down to edge of wire gauze and kept in a petri dish holding sufficient water to remain in content with the bottom of petri dish. After 24-48 hours, the content of a petri dish was emptied into a beaker, diluted to a suitable volume and population counts were made with the help of multi chamber counting dish. Based on the requirement the suspension was diluted with sterile water.

Second stage juveniles were obtained from egg masses collected from heavily infested tomato plants by incubating large number of egg masses at room temperature in water. After 48 hours of incubation the second stage juveniles in water were collected in a 100 ml beaker and volume of water was made up to 50 ml. The nematode suspension was bubbled with the help of 10 ml pipette or ink filler and an aliquot of one ml was transferred to counting dish for counting the juveniles under stereo binocular microscope. Five aliquots were examined from each samples and average population was calculated. For inoculation, larval levels were adjusted with water. So as to add equal volume of nematode suspension in each treatment to give desired inoculum level. The required numbers of juveniles ($1J_2\ g^{-1}$ of soil) in the water were added to potted seedlings to the 2 cm deep holes made on the rhizosphere.

Establishment of *Fusarium* wilt pathogen – root knot nematode interaction in pot culture

Pot culture experiment was conducted with six treatments each replicated four times in a completely randomized block design (CRD) to study the interaction effect of root knot nematode and fungal pathogen on tomato. This experiment was conducted in Rockfeller glass house, Dept. of Plant Pathology TNAU, Coimbatore. The treatments were followed in all the interaction studies are listed below.

Table 1: Establishment of *Fusarium oxysporum* f. sp. *lycopersici* and *Meloidogyne incognita* in pot culture

T. No.	Treatments
T1	Soil inoculation of *Fusarium oxysporum* f. sp. *lycopersici* alone @ 50mg/ kg of pathogen inoculated sand maize medium.
T2	Soil inoculation of *Meloidogyne incognita* alone @ $1J_2$/ g of soil.
T3	Soil inoculation of *F. o.* f. sp. *lycopersici* @ 50mg/kg of pathogen inoculated sand maize medium and soil inoculation of *M. incognita* alone @ $1J_2$/ g of soil (10 days later).
T4	Soil inoculation of *M. incognita* @ $1J_2$/ g of soil and soil inoculation of *F. o.* f. sp. *lycopersici* (10 days later) @ 50mg/ kg of pathogen inoculated sand maize medium.
T5	Soil inoculation of *F. o.* f. sp. *lycopersici* @ 50mg/ kg of pathogen inoculated sand maize medium and soil inoculation of *M. incognita* alone @ $1J_2$/ g of soil (simultaneous).
T6	Un inoculated

Results and Discussion

Characterization of pathogen

The colony colour of *Fusarium* isolates varied from white, white with pinkish white with orange and white with brown tinch. The mycelial topography was raised fluffy growth with central ring and droplets on mycelium. *Fusarium* produced two types of conidia *viz*., micro and macro conidia. Micro conidia were small, oval shaped, hyaline and single or bicelled. The size of micro conidia is 14.78 × 4.27µm. Macroconidia were fusiform, hyaline and multicelled with three to five septa. The size of macro conidia is 36.88 × 5.35µm. The size of chlamydospores is 10.04 × 11.40µm. The number of microconidia was more as compared to macro conidia. Abundant chlamydospores were observed terminally and intercalary. PCR amplification of ITS region of the ten isolates of *F. oxysporum* was performed using the universal primers of forward ITS1 (5'-CTTGGTCATTTAGAGGA AGTAA-3') and reverse ITS 4 (5'-TCCTCCGTTATTGATA TGC-3'). The *Fusarium* genus was amplified as a fragment of 560 bp corresponding to the region of the 18S-28S rRNA intervening sequence for *Fusarium* sp. Then the virulent isolate of *Fusarium* was sequenced and blasted. It showed 99% homology with *F. o.* f. sp. *lycopersici.* Deposited sequence in NCBI and the accession number is MF150001. Hence, the pathogen was identified as *F. o.* f. sp. *lycopersici.*

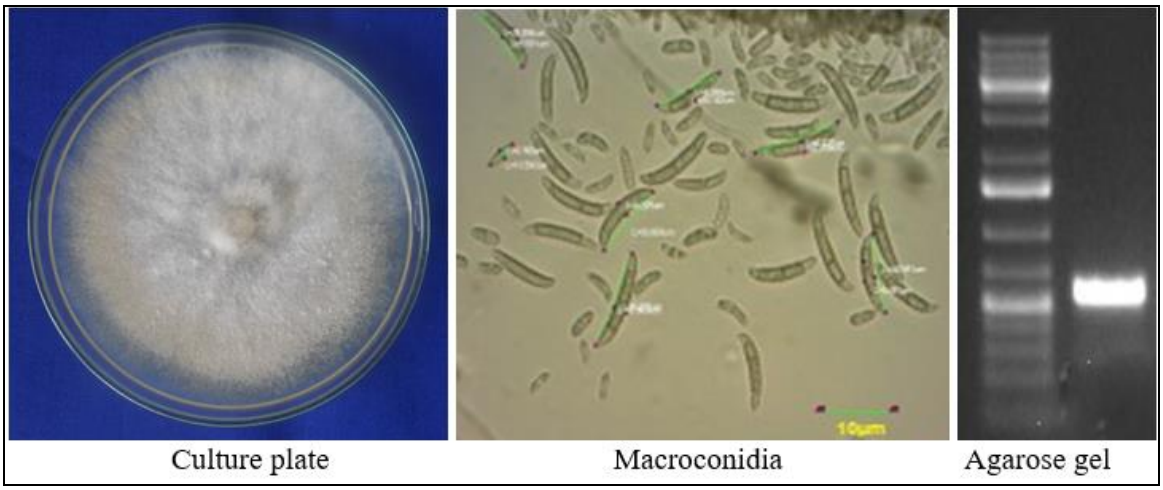

Fig 1: Morphological and molecular characterization of *Fusarium oxysporum* f. sp. *lycopersici*

Extraction and identification of root knot nematode

Extraction of the root knot nematode, *Meloidogyne incognita* was made from infected tomato roots showing typical symptoms of root knot disease, *viz*. chlorotic leaves and premature drying of leaves, stunted growth and knot formation in the roots. Important diagnostic character used for species identification was perinneal pattern or post cuticular pattern found in females of *Meloidogyne*. The perinneal pattern showed high squarish dorsal arch. The lateral ridges were absent, marked by breaks and forks in striae. The striae was coarse, smooth to wavy and the tail had distinct whorl. Based on the observations, the root knot nematode species was characterized as *M. incognita* and used in fungal nematode interaction study.

Fig 2: Fungal and nematode structures on infected host plant

Interaction of *Fusarium* and *M. incognita* in infected plants under glass house

Disease complexes involving nematode and fungi have gained momentum in the recent years leading to considerable yield loss. The data revealed that per cent wilt incidence has significantly increased in all the treatments over control. Maximum wilt incidence was recorded in the treatment where nematode was inoculated 10 days prior to the target pathogen followed by simultaneous inoculation. The treatment in which nematode was inoculated 10 days after target wilt pathogen recorded 50 % wilt incidence.

A maximum of 87.50 % wilt incidence and 53.2 % vascular

infection was recorded in the treatment where nematode was inoculated 10 days prior to the target pathogen. The number of galls recorded in per gram of root was 168.3. This treatment was significantly superior compared to the other treatments in expressing the wilt symptom. This was followed by simultaneous inoculation of both target pathogen and nematode which recorded a wilt incidence of 54.5 % and a vascular infection of 44.6 %.

Table 2: Testing the association of root knot nematode in *Fusarium* wilt severity under glasshouse condition

T. No.	Treatments	Wilt incidence (%)	Vascular infection (%)	Galls present in g^{-1} of roots
T1	Soil inoculation of *F. o.* f. sp. *lycopersici* alone @ 50g kg^{-1} of soil	69.2^{b} (55.60)	42.6^{bc} (40.72)	0.0^{c}
T2	Soil inoculation of *M. incognita* alone @ 1 J_2/g of soil	0.0^{c} (0.52)	0.0^{d} (0.68)	175.5^{a}
T3	Soil inoculation of *F. o.* f. sp. *lycopersici* @ 50g kg^{-1} of soil and *M. incognita* @ 1 J_2/g of soil (10 days later)	50.0^{ab} (43.99)	34.9^{bc} (36.16)	89.53^{b}
T4	Soil inoculation of *M. incognita* @ 1 J_2/g of soil and *F. o.* f. sp. *lycopersici* @ 50g kg^{-1} of soil (10 days later)	87.5^{a} (43.63)	53.2^{a} (46.84)	168.3^{a}
T5	Soil inoculation of *F. o.* f. sp. *lycopersici* @ 50g kg^{-1} of soil and *M. incognita* @ 1 J_2/g of soil (simultaneous)	54.5^{ab} (44.81)	44.6^{b} (41.89)	111.33^{b}
T6	Control	0.0^{c} (0.52)	0.0^{d} (0.68)	0.0^{c}

The number of galls recorded in per gram of root was 111.3. Similar findings were reported by Mahapatra and Swain, (2001) [8] on black gram cv. T-9. Simultaneous inoculation of the host by both the pathogens and nematode inoculation prior to fungus synergistically reduced the plant growth and increase the disease intensity. These effects were less significant when fungus preceded the nematode inoculation. Intensity of the damage caused by the nematode-fungus disease complex was more severe in plants than their separate infection (Jeffers and Roberts, 2003) [5]. Yen *et al.* (2003) [17] reported that *M. incognita* was able to increase the disease incidence of *Fusarium* wilt of watermelon caused *F. oxysporum* f. sp. *niveum* and also decreased resistance ability of watermelon varieties to *Fusarium* wilt. Haseeb *et al.* (2006) [4] exhibited that simultaneous inoculation of both pathogens (*M. incognita* and *F. oxysporum* f. sp. *pisi*) and nematode inoculation 10 days prior to fungus significantly reduced plant growth in banana crop (*Musa paradisiaca* L.) cv. Rasthali. These findings were in conformation with Senthamarai *et al.* (2006) [13]. Simultaneous inoculation of the host by nematode and fungus as well as nematode followed by fungus 15 days later on to *Coleus forskohlii* caused 100 % root-knot disease and significant reduction in plant growth compared to the inoculation by fungus alone or fungus inoculation prior to nematode. Son *et al.* (2009) [14] observed the synergistic effect of *M. incognita* with *Fusarium* wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici.*

The similar results were also observed by Swaransingh *et al* (2010) [15], between

M. incognita and *Macrophomina phaseolina* on Lentil. Inoculation of nematode 7days early prepared the roots for fungus invasion and synergistic effect resulting in increased penetration ability of the fungus. Mallaiah *et al.* (2014) [9] found that inoculation of nematode prior to fungal pathogen recorded the maximum crossandra wilt disease incidence (58.3%). Meena *et al.* (2015) [10] observed that the gerbera wilt disease was found to be more severe with the sequential inoculation of nematodes followed by fungus than with the fungus alone treatment. Lobna *et al.* (2016) [7] found that inoculation of fungus either along with nematode or ten days after the nematode inoculation resulted in significant reduction in fresh weight of shoot and fresh weight of root as well as increase in the tomato wilt incidence compared to plant inoculated with the fungus alone.

Conclusion

The results of the study revealed that inoculation of nematode prior to the inoculation of target pathogen recorded the maximum wilt incidence and confirming the role of nematode as predisposing factor for the entry of the soil borne pathogen *F. oxysporum* f. sp. *lycopersici*, the incident of tomato wilt.

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