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Interaction effect of root knot nematode, *Meloidogyne incognita* and wilt fungus, *Fusarium solani* in cucumber

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Abstract

The interaction between root knot nematode, *Meloidogyne incognita* and wilt fungus, *Fusarium solani* was studied in cucumber under glasshouse condition. A pot experiment was conducted to evaluate the effect and pathogenesis of nematode and fungus individually as well as various combinations viz., inoculation of nematode 7 days prior to fungus, inoculation of fungus 7 days prior to nematode, simultaneous inoculation of nematode and fungus. The results showed that significant reduction of plant growth parameters were observed in combination of nematode inoculated 7 days prior to fungus followed by simultaneous inoculation. Nematode population in the soil, number of galls, number of females and number of egg masses in the roots were higher in the treatment nematode alone followed by nematode inoculated 7 days prior to fungus. Though, nematode reproduction was high in the treatment of nematode alone, the disease incidence was more in nematode inoculated 7 days prior to fungus where the nematode acts as predisposer for other pathogens including fungus. The study was concluded that the effect of nematode inoculated 7 days prior to fungus shows high per cent of wilt disease incidence in cucumber than other combinations.

Keywords: *Meloidogyne incognita*, *Fusarium solani*, nematode wilt disease complex

Introduction

Cucumber (*Cucumis sativus*) is one of the most popular vegetables in India and widely cultivated plant in the gourd family, Cucurbitaceae. It is a creeping vine that bears cucumiform fruits that are used as vegetables. It is grown all over the world due to its high nutritive value and good source of vitamins and minerals. China is the world largest cucumber producer in the world whose production is 64.8 million tons (Food and Agriculture Organization). In India, largest producer Haryana produces 274.40 MT of cucumber during 2017-18 (National Horticulture Board). Continuous growing of cucurbitaceous plants in pandal system makes more disease incidence. Also in polyhouse cultivation, the incidence of disease in cucumber is high. Cucumber plants get affected by several biotic stresses like fungus, bacteria, virus, nematodes and insects. *Fusarium solani*, a soil borne pathogen cause wilt disease in cucumber plants. Root knot nematode, *Meloidogyne incognita* is important and more devastating plant parasite in India ^[1] and it has wide host range of more than 3000 plants including cucurbits. Cucumber is more susceptible to root knot nematodes and *Fusarium* wilt pathogen. Root knot nematode acts as a primary invader of *Fusarium* fungus and cause wilt disease complex. The disease complex was first reported by Atkinson, 1892, where the wilt resistant cotton becomes more susceptible in presence of root knot nematode. Nematode increases the incidence of disease through the entry of other pathogens like fungus and makes disease complex in the plants. A study which observed that root knot nematode, *M. incognita* interacts which *Fusarium solani* produce wilt disease complex in tomato ^[5] and the nematode, *Meloidogyne javanica* interrelated with two soil borne fungus, *Fusarium solani* and *Rhizoctonia solani* which produce disease complex in eggplant ^[10]. Interaction effect of *Meloidogyne incognita* and *Fusarium oxysporum* cause disease severity and more yield loss in black gram ^[3]. Considering the disease complex caused by nematode and pathogens in various crop plants, a research was made to study the interaction effect of *Meloidogyne incognita* and *Fusarium solani* for causing disease complex and its damage intensity in cucumber.

2. Materials and methods

2.1 Isolation of root knot nematode, *Meloidogyne incognita*

The root knot nematodes were isolated from the infested roots of cucumber showing the symptoms of galls or knots. The infested roots are washed in running tap water for 2 to 3 minutes to remove the soil particles adhered on the roots. Then, roots were cut into small pieces and observed for the presence of galls. The females were collected from the galls by teasing out the galls with help of needle under binocular stereo zoom microscope.

2.2 Morphological identification of *Meloidogyne incognita* through Posterior Cuticular Pattern (PCP)

Species of root knot nematodes were confirmed with the help of posterior cuticular pattern through staining of infested roots with acid - fuchsin lactophenol followed by destaining with plain lactophenol for 24 -72 hrs ^[4]. The isolated nematode was confirmed as *M. incognita* through PCP given in the figure. It consists of distinct high dorsal arch composed of smooth to wavy striae. Some striae fork near the lateral lines but distinct lateral incisures are not present ^[4].

2.3 Pure culture of root- knot nematode, *Meloidogyne incognita*

Pure culture of *M. incognita* required for the experimental studies was maintained in the cucumber plants. After confirmation of root knot nematode species, the egg masses were collected from the infested roots of cucumber and placed in a beaker containing distilled water. Then it was allowed to hatch for a week. Twenty days old cucumber seedlings of Hercules hybrid were planted in the earthen pots containing sterile pot mixture. Pot mixture consists of red soil: sand: FYM in the ratio of 2:1:1. Second stage juveniles (J₂) are inoculated near rhizosphere region of cucumber plants maintained in the glasshouse. Egg masses required for the experiment are collected from the roots by uprooting the plants carefully. The egg masses were picked under stereo zoom microscope using forceps and transferred to beaker containing water for hatching which was used for further studies. The nematodes were multiplied and maintained separately as pure culture in the glasshouse, Department of Nematology, Tamil Nadu Agricultural University, Coimbatore.

2.4 Isolation of fungus

In a random survey, infected cucumber plants showing wilted symptoms are collected. The roots were cut into small pieces (0.5-1.0 cm) length, washed thoroughly in fresh water and sterilized with 0.1 % mercuric chloride for 3 min followed by three subsequent washings with sterile distilled water for 3 times and drained the water through sterilized filter paper. Then, the sterilized root bits were placed on the Petri plate containing Potato Dextrose Agar medium. The Petri dishes are incubated at 27±1 °C. After incubation, the pathogen was purified by single hyphal tip method ^[12] and maintained on PDA medium.

2.5 Characterization and pathogenicity of *Fusarium* fungus

2.5.1 Morphological identification of *Fusarium* spp.

The fungus was morphologically identified by taking samples of mycelia fungus from sub cultures. The slides containing fungus was examined under light microscope (Labomed – IVU 5100) and confirmed the presence of microconidia and

macroconidia. From the old cultures, resting spore chlamydospores were also recorded.

2.5.2 Molecular characterization of *Fusarium* spp.

The isolated fungus was characterized by PCR (polymerase chain reaction) method. PCR amplification of ITS region of *Fusarium* isolate was carried out using universal primer pair viz., Internal Transcribed Spacer, ITS 1 (5'-CTTGGTCATTTAGAGGAAGTAA - 3') and ITS 4 (5'-TCCTCCGTTATTGATATGC - 3').

2.6 Mass multiplication of fungus

The pure culture of isolated *Fusarium solani* was maintained on PDA at 27±1 °C. For mass multiplication, the pure cultures of fungus were grown in sterilized sand maize medium in the ratio 9:1 (sand: broken maize). The bags were incubated in the room temperature at 27±1 °C for 15 days.

2.7 Pathogenicity of *Fusarium*

The pathogenic capability of the isolated fungi was carried out under glasshouse condition. Fifteen days old seedlings were planted in the pot containing pot mixture. The isolated test fungus multiplied in the sand maize medium was applied at the rate of 5g/kg of pot mixture. The plants without inoculation of fungus served as a control. The pots were irrigated regularly. The severity of disease and percentage of wilt incidence were recorded after one month of inoculation. Re-isolation was carried out from inoculated plants showing the disease symptoms and the isolated fungus was compared with the original culture used.

2.8 Interaction of *Meloidogyne incognita* and *Fusarium solani* in cucumber under glasshouse condition.

A pot culture experiment was conducted under glasshouse condition to study the interaction effect of root knot nematode and wilt pathogen. The experiment was laid out in a completely randomized block design (CRD) replicated four times with six treatments. Earthen pots of 3 kg capacity were filled with sterilized pot mixture and surface sterilized hybrid seeds of cucumber, Hercules were sown and 25 days old seedlings were transplanted in each pot at one plant per pot. The treatments were i. *M. incognita* alone ii. *Fusarium solani* alone iii.

M. incognita inoculated 7 days prior to *Fusarium solani* inoculation. iv. *Fusarium solani* inoculated 7 days prior to *M. incognita* inoculation v. simultaneous inoculation of *M. incognita* and *Fusarium solani* vi. Uninoculated control. The second stage juveniles of *M. incognita* were inoculated @ 1000 J₂/kg of soil and *F. solani* inoculated at the rate of 5g/kg of soil. After 60 days of inoculation, observations were made on root population of nematode include no. of. galls, egg masses and eggs/egg masses, gall index of 1-5 scale (Root knot index: 1 = 0 galls; 2 = 1-25 galls; 3 = 26-50 galls; 4 = 51-75 galls; 5 = >75 per cent galls per root system) ^[6]. Nematode populations in soil were assessed by Cobb's decanting sieving method ^[2] followed by Modified Baermann funnel technique. Per cent wilt incidence due to fungus was recorded using number of wilt infected plants/total number of plants taken for observation.

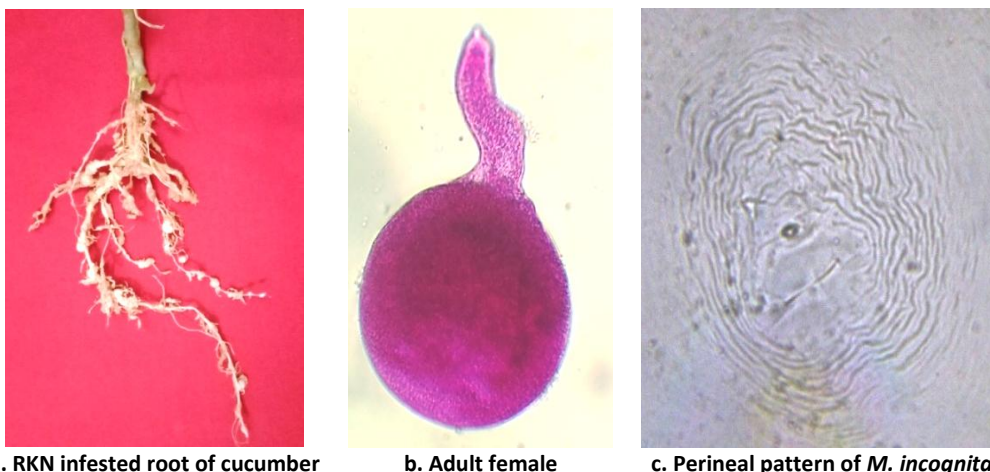
3. Results

3.1 Identification of nematode species associated with cucumber

Meloidogyne species collected from cucumber was identified

by cuticular markings present in the perineal area of matured female. The PCP were observed with high dorsal arch

composed of smooth to wavy striae (Plate 1) and it is confirmed as *Meloidogyne incognita*.



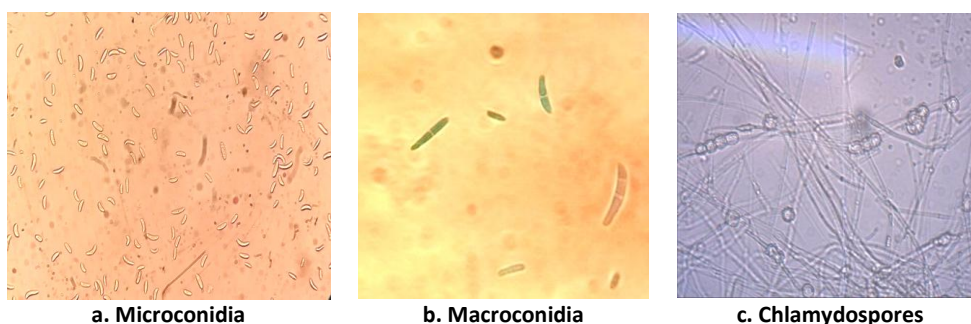
a. RKN infested root of cucumber b. Adult female c. Perineal pattern of *M. incognita*

Plate 1: Morphological identification of Root knot nematode

3.2 Identification of *Fusarium* species associated with cucumber

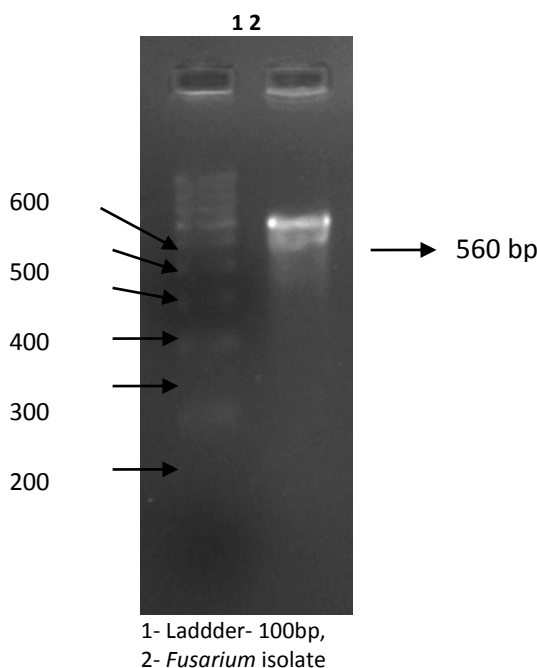
The fungus was confirmed as *Fusarium* by its conidial structures and microspores, macrospores and chlamydospores production of fungus (Plate 2). PCR was performed to identify the *Fusarium* spp. using universal primers ITS 1 and ITS 4. The results revealed that, the isolates were amplified

with amplicon size of approximately 560 bp corresponding to 18S-28S rDNA region (Plate 3). The PCR product were resolved on 1.2 % agarose, purified and sequenced at Barcode Biosciences, Bangalore, India. The sequence analysis of an isolate revealed that it had nucleotide sequence identity 99.5% homology with *Fusarium solani*. Thus the pathogen causing wilt disease in cucumber was confirmed as *Fusarium solani*.



a. Microconidia b. Macroconidia c. Chlamydospores

Plate 2: Morphological identification of *Fusarium* spp



1- Ladder- 100bp,
2- *Fusarium* isolate

Plate 3: Molecular characterization of *Fusarium* species

3.3 Interaction effect of *Meloidogyne incognita* and *Fusarium solani*

The interaction effect of *M. incognita* and *F. solani* on cucumber was studied under glasshouse condition and the results furnished on table 1. The results indicates that parameters, nematode inoculated 7 days prior to fungus having significantly reduced plant height (96.50 cm) followed by the treatment concomitant inoculation of fungus and nematode which was found to be 105.25 cm when compared to control (152.25 cm). Minimum root length was observed in nematode inoculated prior to fungus (18.25 cm) and followed by simultaneous inoculation of nematode and fungus (21.00 cm) compared to control (43.75 cm). Minimum fresh shoot weight (15.43 g), dry shoot weight (8.25 g), fresh root weight (3.58 g) and dry root weight (1.73 g) also highly reduced in the treatment, nematode inoculation prior to the fungus than

control.

The data represented in the table 2 revealed that number of juveniles per 250 g of soil (344.75), number of females per 5 g of root (74.5) and number of egg masses per 5g of root (52.5) were maximum in the treatment nematode alone. It was followed by the treatment nematode inoculated 7 days prior to fungus where number of Juveniles/250 g of soil was 323.25, number of females/5g of root was 56.75 and number of egg masses/5g of root was 42.00. There were no nematode juveniles, females and egg mass in the treatment fungus alone and untreated control. The wilt incidence has significantly increased in all the treatments compared to the control. The maximum wilt incidence (100%) was recorded in the nematode inoculated 7 days prior to the fungal pathogen followed by concomitant inoculation of *M. incognita* and *F. solani* which was recorded 75% incidence.

Table 1: Interactive effect of *Meloidogyne incognita* and *Fusarium solani* on plant growth parameters in cucumber

Treatments	Plant growth parameters					
	Shoot			Root		
	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)
T ₁ - Nematode alone	124.50 ^e (18.23)	28.85 ^d (35.78)	14.38 ^e (51.47)	35.00 ^c (20.00)	8.28 ^d (46.78)	5.18 ^c (52.55)
T ₂ - Fungus alone	119.25 ^d (21.67)	27.08 ^d (39.73)	13.25 ^d (55.27)	29.25 ^b (33.14)	7.45 ^{cd} (52.09)	4.35 ^b (60.27)
T ₃ - Nematode inoculation 7 days prior to fungus	96.50 ^a (36.62)	15.43 ^a (65.66)	8.25 ^a (72.15)	18.25 ^a (58.29)	3.58 ^a (77.01)	1.73 ^a (84.20)
T ₄ - Fungus inoculation 7 days prior to nematode	113.00 ^c (25.78)	23.90 ^c (46.80)	11.38 ^c (61.60)	26.75 ^b (38.86)	6.65 ^c (57.23)	3.35 ^b (69.40)
T ₅ - Simultaneous inoculation nematode and fungus	105.25 ^b (30.87)	19.03 ^b (57.65)	9.38 ^b (68.35)	21.00 ^a (52.00)	4.55 ^b (70.74)	1.85 ^a (83.10)
T ₆ - Uninoculated control	152.25 ^f	44.93 ^e	29.63 ^f	43.75 ^d	15.55 ^e	10.95 ^d
CD (p= 0.05)	2.55	0.72	0.53	0.73	1.98	1.24
SEd	1.20	0.34	0.25	0.34	0.9	0.59

*Values are mean of four replications

**Values in parantheses are percent decrease over control

Table 2: Interactive effect of *Meloidogyne incognita* and *Fusarium solani* on nematode reproduction in cucumber

Treatments	Nematode reproduction				Wilt disease incidence (%)
	J ₂ / 250g of soil	Female/ 5g of root	Egg mass/ 5g of root	Root knot index	
T ₁ - Nematode alone	344.75 ^a (18.57)	74.50 ^a (8.66)	52.5 ^a (7.27)	5.00	0.00
T ₂ - Fungus alone	0.00 ^e (0.71)	0.00 ^e (0.71)	0.00 ^d (0.71)	0.00	50.00
T ₃ - Nematode inoculation 7 days prior to fungus	323.25 ^b (17.99)	56.75 ^b (7.56)	42.00 ^b (6.50)	4.00	100.00
T ₄ - Fungus inoculation 7 days prior to nematode	282.25 ^d (16.81)	25.50 ^d (5.09)	25.00 ^c (5.04)	2.75	50.00
T ₅ - Simultaneous inoculation of nematode and fungus	302.50 ^c (17.41)	34.75 ^c (5.93)	41.50 ^b (6.48)	3.50	75.00
T ₆ - Uninoculated control	0.00 ^e (0.71)	0.00 ^e (0.71)	0.00 ^d (0.71)	0.00	0.00
CD (p= 0.05)	0.40	0.38	0.49		
Sed	0.19	0.18	0.23		

*Values are mean of four replications

**Values in parentheses are square root transformed values.

4. Discussion

The results of the study revealed that the plant growth was affected in all treatments where the nematode and fungus inoculated to the plant both individually and in combination. But the incidence of disease complex was significantly higher in the treatment where the *M. incognita* inoculated 7 days prior to *F. solani* and shows more synergistic followed by the

treatment where both the pathogens inoculated simultaneously. Similar effect was also observed in the protected cultivation of cucumber where the nematode *M. incognita* inoculated 7 days prior to the fungus *Fusarium oxysporum f. sp. cucumerinum* which shows high level of disease incidence followed by concomitant inoculation of nematode and fungus than the control [9] and the maximum

wilt incidence was observed in the treatment where the *Meloidogyne incognita* was inoculated 15 days prior to the inoculation of *Fusarium solani* in Bittergourd under pot culture experiment ^[11]. Thus the nematode acts as a primary invader for causing disease complex. It was proved in the study Meena *et al.* ^[8] that the nematode acts as a predisposer for the spread of secondary fungal pathogens. The wilt disease was caused by interaction of root knot nematode, *Meloidogyne incognita* with wilt fungus *Fusarium solani* in Gerbera.

Although the individual pathogen can reduce plant growth, combined effect of nematode and fungus resulted increased the severity of disease. It was proved in the study Kalaivanan *et al.* ^[7], where the sequential and concomitant inoculation of the root knot nematode, *Meloidogyne incognita* and fungus, *Sclerotinia sclerotiorum* cause more disease severity than the individual effect of nematode and fungus. In this study, the treatment receiving nematode inoculation 7 days prior to fungus shows higher reduction in plant growth parameters and nematode population in soil and root and its reproduction. *M. incognita* acts as a predisposer of *F. solani* which enhances the disease development in cucumber. But the wilt incidence was higher in nematode inoculation prior to fungus and concomitant inoculation of fungus and nematode. Thus, present study proved that presence of nematode paves ways for the entry of fungal pathogens which increases wilt disease severity than the individual inoculation of nematodes and fungus in cucumber.

5. Conclusion

The present investigation concludes that treatment of root knot nematode *Meloidogyne incognita* inoculated 7 days prior to the inoculation of *Fusarium solani* causes maximum per cent wilt disease incidence compared to control. The nematode acts as a primary invader for spread of fungal pathogen. This interaction enhances incidence of wilt diseases and reduce plant growth parameters in cucumber under polyhouse condition. Thus, the study proved that the nematode paves way for entry of fungal pathogens to increase the wilt disease severity in cucumber.

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