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Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* on vegetable cowpea (*Vigna unguiculata* (L.) Walp

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ABSTRACT: Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* in vegetable cowpea, *Vigna unguiculata* was studied at the Department of Nematology, College of Agriculture, Vellayani during 2019-2021. Highest cohabitation of both the pathogens was observed when *M. incognita* was inoculated seven days prior to *F. oxysporum* inoculation. Presence of nematodes prior to fungus caused early disease incidence (23 days) and increased severity of wilt disease. Highest reproduction factor (2.34) was observed in *M. incognita* alone followed by *M. incognita*+ *F. oxysporum* one week after nematode inoculation (1.79). Nematode reproduction and galling showed significant reduction in simultaneous inoculation of both pathogens compared to nematode inoculation one week prior to fungus inoculation. Plants inoculated with *M. incognita* + *F. oxysporum* at one week after nematode inoculation showed significant reduction in growth parameters viz. shoot length (55.40 per cent), fresh weight of shoot (63.44 per cent) and root (49.01 per cent) compared to unincoculated control plants. Lowest number of nodules/5g root (14.00) was recorded in plants treated with *M. incognita* alone followed by *M. incognita*+ *F. oxysporum* one week after nematode inoculation (19.30).

Keywords: Vegetable cowpea, *Meloidogyne incognita*, *Fusarium oxysporum,* interaction

INTRODUCTION

Vegetable cowpea, *Vigna unguiculata* sub sp. *sesquipedalis* (L.) Walp is one among the most cultivated vegetables in Kerala. It is an extensively adapted, stress tolerant vegetable crop produced worldwide in warm to hot regions. Cowpea is cultivated as a vegetable in India, mostly in semi-arid and dry regions, with an area of 654 lakh hectares, a productivity of 916 kg/ha and a production of 599 lakh tonnes (Joshi, 2018). The increasing economic relevance of the crop is owing to its food value as it is good source of protein, vitamin A, iron, phosphorus and potassium. The ability of cowpea to fix atmospheric nitrogen through biological nitrogen fixation enhances soil fertility. Root knot nematode (RKN) *Meloidogyne incognita* (Kofoid and White) Chitwood is one of the main biotic constraints which reduces the quantity and quality of the crop throughout the world. Symptoms of *M. incognita* infection include stunting, yellowing, wilting and galling of roots. The average loss caused by root knot nematode in cowpea is 14.6 per cent in India (Mahantheshwara *et al*., 2020). Besides physiological changes in root, root-knot nematodes act as predisposing agents for entry of many pathogenic organisms like fungi, bacteria and virus. Fusarium wilt (FW) caused by the fungus *Fusarium oxysporum* f. sp. *tracheiphilum* (Fot) is one of the main threats to cowpea production throughout world. Plants show vascular streaks, basal swelling of stem, partial wilting in early stages and complete wilting in later stages leading to ultimate death. The disease complex caused by *M. incognita* and *F. oxysporum* occurs most frequently in vegetable cowpea in Kerala. Disease complexes are formed when two organisms invade a crop at the same time, resulting in major yield losses. The mechanical wounding and the resistance breaking ability of nematode favor the establishment of the fungus. The disease affected plants show brown streaks in vascular system resulting in physiological and biochemical alterations (Troisi *et al*., 2010). The objective of the present study was to assess the interaction between *M. incognita* and *F. oxysporum* in vascular wilt disease of cowpea.

MATERIALS AND METHODS

Identification of nematode and preparation of nematode inoculum

Identification of root-knot nematode was done by observing perineal pattern of adult females (Taylor and Netscher, 1974). Vegetable cowpea plants infected with root-knot nematodes were collected from Instructional Farm, Vellayani and washed thoroughly under running water. Mature females (10-15) were teased from galled roots and kept in a glass slide. They were cut at neck region and internal contents were pushed out by pressing. A cut was given in posterior region and the cuticle was placed in 45% lactic acid for cleaning the remaining body tissues. It was trimmed further under stereoscopic binocular microscope until it acquired square shape. This

Table 1. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* on growth parameters of vegetable cowpea under pot culture condition

Treatments	Shoot*						Root*				No of nodules in root (5 g)*	% reduction over untreated
	Length (cm)	% reduction over control	Fresh weight (g)	% reduction over untreated	Dry weight (g)	% reduction over untreated	Fresh weight (g)	% reduction over untreated	Dry weight (g)	% reduction over untreated		
T1	21.10[b]	15.60	6.63[b]	9.55	3.84[b]	25.87	5.40[a]	19.21	3.33[a]	28.23	14.00 (3.73)[f]	72.55
T2	19.33[c]	22.68	5.13[c]	30.01	3.04[c]	41.31	4.15[c]	8.39	2.07[c]	13.39	36.30 (6.02)[b]	28.82
T3	18.26 [d]	26.96	4.38[d]	40.25	2.44[d]	52.90	3.68[d]	18.76	1.67[d]	30.13	23.30 (4.82)[d]	54.31
T4	11.15[f]	55.40	2.68[f]	63.44	1.12[f]	78.38	2.31[f]	49.01	0.98[f]	59.00	19.30 (4.39)[e]	62.16
T5	15.43[e]	38.28	3.73[e]	49.11	1.95[e]	62.36	2.89[e]	36.20	1.25[e]	33.63	31.00 (5.56)[c]	39.22
T6	25.00[a]	-	7.33[a]	-	5.18[a]	-	4.53[b]	-	2.39[b]	-	51.00 (7.16)[a]	-
CD (0.05)	**0.705**		**0.640**		**0.116**		**0.190**		**0.618**		**(0.359)**	

T1- *M. incognita* alone; T2- *F. oxysporum* alone; T3- Simultaneous inoculation of *M. incognita* and *F. oxysporum*; T4- *M. incognita* + *F. oxysporum* one week after nematode inoculation; T5- *F. oxysporum*+ *M. incognita* one week after fungus inoculation; T6- Uninoculated control with neither nematode nor fungus.

Figures given in parenthesis are square root transformed values *Mean of four replications

was transferred to a drop glycerine on a clean side and observed under microscope after placing cover glass over glycerine drop.

Pure culture of root-knot nematode was maintained in glass house of Department of Nematology, College of Agriculture, Vellayani by collecting egg masses from infected cowpea plants. Collected egg masses were surface sterilized using 0.1% sodium hypochlorite for 3 min and 95% ethanol for 1 min and later washed in sterile water for three times and placed in distilled water for hatching. Newly hatched second stage juveniles were inoculated in twenty five day old tomato seedling variety Vellayani Vijay @two juveniles/g soil for maintenance of culture.

Identification of fungal pathogen and mass multiplication

F. oxysporum was isolated from diseased vegetable cowpea plants from Instructional farm, Vellayani by direct plating method and maintained as pure culture in PDA at 25±2 ^{0}C. Growth of the fungus was observed at seven and ten days after inoculation and identified based on conidial characters. DNA extracted was subjected to PCR amplification using universal primers ITS1 and ITS4 (White *et al*., 1990) for molecular characterization.

Mass multiplication of fungal pathogen was done in sand : maize mixture (9:1) (Lewis and Papavizas, 1984). For the preparation of 100 g of sand maize mixture, 90 g sand and 10 g corn flour were mixed and 20 mL of distilled water was added. The mixture was filled into 250 mL conical flasks and plugged with cotton and sterilized in an autoclave at 121^{o}C and 15 k Pa pressure for 20 min. Fungal discs of size 7 mm were taken from seven days old culture plates using cork borer. 10 to 12 bits were added to each conical flask and the fungus was allowed to multiply for 15 days under room temperature. After 15 days, the sand-maize media was observed to be completely covered with white mycelial growth of fungus.

Interaction studies of nematode- fungus in vegetable cowpea

Pot culture experiment was conducted in glass house of Department of Nematology, College of Agriculture, Vellayani during 2020-21. Nematode culture was maintained at Nematology glass house, College of Agriculture, Vellayani. Earthern pots of size 36x20x20'' were filled with denematized potting mixture containing soil, sand and farm yard manure in 2:1:1 proportion and seeds of vegetable cowpea (variety Vellayani Jyothika) were sown with six treatments and four replications. Inoculation of both pathogens (*M. incognita* and *F. oxysporum* multiplied in sand maize medium (20×10 ^{7}cfu/g) @500g) was done in the rhizosphere of 25 days old cow pea seedlings either alone, simultaneously or sequentially. Nematodes were inoculated @1 juvenile/g soil. The treatments were T1- *M. incognita* alone, T2- *F. oxysporum* alone, T3- Simultaneous inoculation of *M. incognita* and *F. oxysporum,* T4- *M. incognita* + *F. oxysporum* one week after nematode inoculation, T5- *F. oxysporum*+ *M. incognita* one week after fungus inoculation, T6- Control with neither nematode nor fungus.

Observations were recorded on plant growth parameters (shoot length, dry and fresh weight of shoot and root), nematode population characteristics (nematode population in soil (200cc), root (5g), number of females (5g root), number of galls (5g root), egg masses (5g root), eggs per egg mass, disease incidence, days taken for initiation of disease) and number of nodules (5g root). Nematode population in soil was estimated by Cobb's sieving and decanting method and modified Baermann's funnel technique. Reproduction factor was calculated by formula given by Oostenbrink (1966) and disease incidence was documented as per method developed by Singh (2002). Data generated from the experiment were subjected to analysis of variance (ANOVA) test (Cochran and Cox, 1965). Those variables which did not satisfy the basic assumptions of ANOVA were subjected to angular or square root transformation.

RESULTS AND DISCUSSION

Identification of *Meloidogyne* species associated with vegetable cowpea

Perineal pattern of the collected females was identified as *M. incognita* based on identification key (Eisenback, 1985) that include high dorsal arch which were flattened at the top and striae distinct and wavy.

Identification of *Fusarium* species associated with vegetable cowpea

Fusarium species was identified based on morphological characters *viz.* purplish tinged colony with white mycelia as well as presence of macro and micro conidia. Micro conidia were elliptical in shape and macro conidia possessed 4±1.5 septation. The DNA of the internal transcribed spacer regions (ITS) were amplified using the universal primers ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC3'). Results of the blast analysis showed homology with matching sequence of *F. oxysporum* in NCBI data base.

Effect of nematode pathogen interaction on growth parameters of vegetable cowpea

There was significant reduction in growth parameters of cowpea plant when nematode and fungus were inoculated at different intervals compared to uninoculated control (Table 1). Inoculation of nematodes one week

Table 2. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* on nematode population characteristics in vegetable cowpea under pot culture condition

Treatments	Nematode population* (30 DAI)			Reproduction factor (RF=Pf/Pi)*	No. of galls (5 g)*	No. of egg masses (5 g)*	No. of eggs in egg mass
	Soil (200 cc)	Root (5 g)	No. of females (5 g)				
M. incognita alone	573.30 (23.95)[a]	58.30 (7.70)[a]	40.66 (6.41)[a]	2.34[a]	66.66 (8.22)[a]	74.00 (8.63)[a]	256.00 (16.03)[a]
F. oxysporum alone	0.00 (1.00)[e]	0.00 (1.00)[e]	0.00 (0.70)[e]	0.00[e]	0.00 (1.00)[e]	0.00 (0.70)[e]	0.00 (0.70)[e]
Simultaneous inoculation of *M. incognita* and *F. oxysporum*	490.00 (22.15)[c]	41.30 (6.50)[c]	20.00 (4.52)[c]	1.46[c]	42.33 (6.58)[c]	52.30 (7.26)[c]	208.60 (14.46)[c]
M. incognita + *F. oxysporum* at one week after nematode inoculation	551.33 (23.50)[b]	51.30 (7.23)[b]	30.66 (5.58)[b]	1.79[b]	56.66 (7.59)[b]	67.30 (8.23)[b]	239.00 (15.47)[b]
F. oxysporum + *M. incognita* at one week after fungus inoculation	433.00 (20.83)[d]	34.3 (5.56)[d]	15.00 (3.93)[d]	1.14[d]	22.33 (4.82)[d]	40.00 (6.36)[d]	122.60 (11.09)[d]
Uninoculated control	0.00 (1.00)[e]	0.00 (1.00)[e]	0.00 (0.70)[e]	0.00[e]	0.00 (1.00)[e]	0.00 (0.70)[e]	0.00 (0.70)[e]
CD (0.05)	**(0.217)**	**(0.277)**	**(0.201)**	**(0.067)**	**(0.175)**	**(0.190)**	**(0.461)**

Pf- Total nematode population (soil+root+ number of females); Pi-Initial nematode population

Figures given in parenthesis are square root transformed values *Mean of four replications

prior to fungus recorded lowest growth parameters of plants (shoot length-11.15 cm, fresh shoot weight-2.68 g, dry shoot weight-1.12 g, fresh root weight-2.31g and dry root weight-0.98 g). Plants inoculated with *M. incognita* + *F. oxysporum* at one week after nematode inoculation showed 55.40, 63.44 and 78.38 percentage reduction in length, fresh weight and dry weight of shoot compared to unincoculated control plants. This finding in this study is in agreement with Samuthiravalli and Sivakumar (2008) who observed that when *M. incognita* combined with fungus *F. oxysporum* f. sp. *lycopersici*, the impact was amplified due to synergism and growth was suppressed in tomato plants, when compared to that with fungal inoculation alone. The highest synergistic effect in sequential inoculation of nematode and pathogen noticed in this study may be attributed to the fact that root knot nematodes enhanced the occurrence, pace of development and severity of wilt resulting in lowering of plant growth indices like plant length, shoot and root weight by providing entry sites for the fungal pathogen (Malhotra *et al*., 2011). Similar trend was observed in the case of fresh weight and dry weight of root also (49 to 59 per cent reduction over control). Meena *et al.* (2016) reported that prior inoculation of nematodes in carnation disrupted the vascular tissues and reduced the transportation of water and nutrients to foliar systems resulting in reduced photosynthetic rate and chlorophyll content in plants. In the present study also, sequential inoculation of nematodes prior to fungus modified host root physiology and caused synergistic effect in reducing plant growth than their individual infection. Lowest number of nodules was recorded in plants inoculated with *M. incognita* alone with 72.55 per cent reduction over uninoculated control. This might be due to the reduction in the capacity of rhizobia bacterial strains to fix nitrogen, especially when the plants are affected with specific pathogens like root knot nematodes. This result corroborated with Izuogu *et al*. (2019), who recorded a reduction in the number of nodules in cowpea plants inoculated with nematode. *M. incognita* infection and gall formation disrupted physiological processes of plants which decreased rhizobium activity in the nodular tissue, resulting in poor nodulation and reduced nitrogen fixation.

Effect of nematode-pathogen interaction on nematode population characteristics

Data presented in Table 2 revealed that there was significant difference in nematode population and disease parameters when both the pathogens were inoculated singly or in combination at different intervals. The highest nematode multiplication was recorded in plants inoculated with *M. incognita* alone followed by *M. incognita* seven days prior to *F. oxysporum* inoculation. *M. incognita* alone inoculated plants recorded highest population of nematodes in soil (573.30/200cc) 30 days after nematode inoculation (DAI) followed by *M. incognita* + *F. oxysporum* one week after nematode inoculation (551.33). Similar trend was observed in root also. This finding is in line with that of Husain and Salman (2019) who reported that nematode population in soil at

Table 3. Effect of *Meloidogyne incognita* and *Fusarium oxysporum* on disease incidence in vegetable cowpea

Treatment	Disease incidence (%)*	Disease index (%)*	Days taken for initiation of disease*
M. incognita (alone)	0.00 (2.86)^{d}	0.00 (2.86)^{d}	No disease
F. oxysporum (alone)	33.33 (35.20)^{c}	25.00 (29.16)^{c}	29
Simultaneous inoculation of *M. incognita* and *F. oxysporum*	33.33 (35.20)^{c}	29.16 (27.57)^{c}	27
M. incognita + *F. oxysporum* at one week after nematode inoculation	100.00 (90.00)^{a}	91.66 (76.17)^{a}	23
F. oxysporum + *M. incognita* at one week after fungus inoculation	66.60 (54.76)^{b}	45.83 (42.59)^{b}	26
Uninoculated control	0.00 (2.86)^{d}	0.00 (2.86)^{d}	No disease
CD (0.05)	**(4.419)**	**(8.920)**	

Figures in parenthesis are arc sine transformed values *Mean of four replications

harvest increased by four folds than its initial population in the plants inoculated with *M. incognita* alone in tomato. This might be due to the fact that the interaction between the nematode and fungus is not helpful always for nematode multiplication as the secondary pathogen may penetrate and feed on nematode feeding sites, causing nematode famine and death. Highest number of galls (66.66/5g root), females (40.66/5g root), egg masses (74.00/5g root) and eggs in egg mass (256.00/5g root) also observed in plants inoculated with *M. incognita* alone. Significant reduction in number of galls (56.66/5g root), females (30.66/5g root), egg masses (67.30/5g root) and eggs/egg mass (239.00/5g root) was observed in plants inoculated with *M. incognita* seven days prior to *F. oxysporum* inoculation. These observations were in agreement with the study conducted by Al-Hazmi and Al-Nadary (2015) who mentioned that inoculation of nematode prior to the fungus in green beans caused anatomical and physiological changes in root leading to formation of root galls and predisposing the plant to fungal infection and its establishment which caused the deleterious effect to nematodes resulting in reduction in their number. Gall formation by nematode was affected due to the attack of fungal mycelia on nematode cells and toxic metabolites produced by the fungus caused decrease in number of juveniles, thus reducing the formation of galls on roots (Askary, 2015). Reproduction factor was highest (2.34) in the plants treated with *M. incognita* alone followed by *M. incognita* at seven days prior to *F. oxysporum* inoculation (1.79). This decrease may be due to the development of mycelial mat on roots following fungal invasion, which created adverse conditions for the developing nematodes causing their sex reversal and formation of males that leave the roots (Hajji-Hedfi *et al.,* 2017).

Effect of nematode-pathogen interaction on disease incidence in vegetable cowpea

The highest disease severity percentage (100.00 %) and early onset of the disease (23 days) were observed in the plants inoculated with *M. incognita* at seven days prior to *F. oxysporum* inoculation (Table 3). This enhancement in disease might be due to the interaction between both the pathogens in rhizosphere. Similar findings were reported by Ramalingam (2019) in tomato and Sanjeevkumar *et al*. (2018) in the banana cv. Monthan. They reported that exudates liberated from *M. incognita* infected plants enhanced the germination of *Fusarium* propagules present in soil. It also hindered the activity of actinomycetes, which inhibit the wilt fungus, in turn allowing *Fusarium* pathogen to grow without any curb. Higher reserve material found in the nurse cells formed by nematodes helped fungal pathogen to grow and invade rapidly on the nutritionally dense giant cells. Disease index was least (25.00) *F. oxysporum* alone than other treatments. The reduction in disease incidence may be due to the late or delayed entry of fungus into the plant because of the absence of predisposing agent i.e., nematode. This finding is in line with that of Haseeb *et al*. (2007) who reported that there was an increase in disease when *M. incognita* was inoculated prior to *F. oxysporum* f. sp. *pisi* in garden pea. The present investigation of *M. incognita* and *F. oxysporum* on vascular wilt disease complex in cowpea clearly demonstrated prior inoculation of nematodes reduced the incubation period of fungus, thereby resulting earlier wilting symptoms. Inoculation of nematode seven days prior to fungus inoculation recorded the maximum disease incidence, confirming the role of *M. incognita* as predisposing factor for the entry of the soil borne pathogen *F. oxysporum* in vegetable cowpea.

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