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Interaction between *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lycpersici* on Tomato

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A B S T R A C T

The interaction between root knot nematode *Meloidogyne incognita* and the fungus *Fusarium oxysporum f.sp.lycopersici* was studied on tomato cultivar Pusa Ruby under pot house conditions. The effect of the nematode in combination with the fungus enhanced the suppression of plant growth than that of the fungus alone. Inoculation of the nematode and fungus exhibited a synergistic effect on growth retardation of plants. Significant reduction in plant heights in pots with nematode alone (43.77 cm) and fungus alone (43.83 cm) were also recorded, as compared to un-inoculated control (56.23 cm).

Introduction

Tomato (*Solanum lycopersicum L.*) is one of the most popular vegetable crops grown in the world, next to potato. It is used as a fresh vegetable and also can be processed and canned as a paste, juice, sauce, powder or as a whole (Barone and Frusciante, 2007). The ripe fruits are good source of vitamin A, B and C which add wide varieties of colour and flavour to the food. Recently, it started gaining more medicinal value because of the antioxidant property of ascorbic acid and lycopene content (Anon, 2002). In India, it is being grown in about 4.97 lakh hectares with an annual production of 10, 260, 600 metric tonnes. India ranks as the fourth country in the world in tomato production after China, United States and Turkey. Area under tomato cultivation in India is about 7.3 per cent of the total cropped land with 18227 million tonnes production (Anon., 2014). A number of economically important diseases of tomato caused by fungi are transmitted by seed or transplants. Tomatoes are parasitized by a number of pathogens, including *Fusarim oxysporium* f.sp. *lycopersici* (Sacc. W.C. Snyder *et al.*, 2003) the causal agents of fusarium wilt, which is one of the most important pathogen of as tomato pathogen (Jones *et al.*, 1982). Due to high temperature and humidity, *Fusarium oxysporum*

f.sp.*lycopersici* can cause significant damage. *Fusarium oxysporum* f.sp. *lycopersici* is soil borne pathogen which can persist for many years in the soil without host. Most infections originate from the population associated with infected tomato debris. Healthy plants can become infected by *Fusarium oxysporum* if the soil, is infested with the pathogen (Farr *et al.,* 1989). Browning of the vascular tissue is strong evidence of Fusarium wilt (Snyder and Hans, 2003). The Root knot nematode, *Meloidogyne incognita* is important pest of vegetables in subtropical and tropical climates including india and infict significant yield losses (Akram and Khan, 2006). *Meloidogyne incognita* is the most dominant species accounting for 64 per cent of total population which is widely prevalent inflicting serious loss to tomato fruit yield (Sasser, 1980). The fungus also develops synergistic relationship with Meloidogyne species leading to root-knot wilt disease complex (Patel *et al.,* 2000).

Materials and Methods

Tomato susceptible cultivar Pusa Ruby was used for the present studies. The fungus was grown on corn meal medium as per the method described earlier was subsequently autoclaved at 1.05 kg/cm_2 for two hours before use. Ten cm earthen pots holding 500g of soil were used in the present studies. A constant level of 1000 second stage juveniles was inoculated per pot as per treatment. The technique for extraction and disinfection of these nematodes was the same as described in earlier. The nematodes were pipetted around the seedlings. Due precautions were observed to avoid contamination from one pot to another. Wherever, the nematodes were to be inoculated, one week after, three glass rods were fixed two cm deep in a circle two cm in diameter. At the time of inoculation, the glass rods were removed and the nematode suspension was evenly distributed in the holes and these were plugged with sterile soil.

The Inoculating soil with fungus, *Fusarium oxysporum*, 50g content of each flask containing corn meal inoculated with the fungus was mixed in each pot for fungus, fungus + nematode one week after, and simultaneous inoculation of fungus and nematode. The other pots which were to be inoculated by nematode, nematode and fungus, fungus one week after, and control were mixed with uninoculated corn meal. When the fungus was to be inoculated one week after nematode inoculation, the technique suggested by Grewal and Pall (1974) was adopted with slight modification of placing three glass rods equidistant in a circle of two cm diameter and plugging the holes with sterile soil after introducing the actively growing *Fusarium oxysporum* derived from the corn meal. The treatments consisted of (a) control without fungus and nematode (p) nematode alone (N), (c) Fungus alone (F), (d) simultaneous inoculation of nematode and fungus (NF), (f) nematode at the time of sowing and fungus one week after (N 1 F2) fungus at the time of sowing and nematode after one week (F1 N2). A total of 30 pots were thus randomized over the glass house bench following CRD and watered daily with an equal quantity of sterilized distilled water if when required. The experiment was concluded after 45 days after Inoculation. The glass house temperature during the course of experiment was ranged from 27 to 34^0 C.

Results and Discussion

The effect of cohabitation of *M. incognita* and *F. oxsysporum* on tomato was studied under pot conditions and the results are presented in table 1. The data indicated that where the nematode inoculated first and fungus a week after reduced (28.07 cm) plant height to a significant level when noted compared to control (56.23 cm) and rest of the treatments. This was followed by the treatment where

nematode and fungus inoculated simultaneously. The plant height in this treatment was noted to be (29.97 cm) where as the treatment in which fungus inoculated first and nematode a week after showed 31.07 cm plant height. The plant heights in both these treatments remained statistically at par. Significant reduction in plant heights in pots with nematode alone (43.77 cm) and fungus alone (43.83 cm) were also recorded, as compared to un-inoculated control (56.23 cm).

Minimum root length (6.93 cm) was observed that where the nematode inoculated first and fungus a week after followed by the treatment where nematode and fungus inoculated simultaneously (8.30 cm). Significant reduction in the root lengths were observed where nematode alone (10.90 cm) and fungus alone (11.50 cm) were recorded, as compared to un-inoculated control (12.67 cm). Minimum (0.66 g) weight of fresh shoot was noted with nematode inoculated first and fungus seven days after followed by nematode and fungus inoculated simultaneously (0.75 g) and fungus inoculated first and nematode a week after (1.40 g). The fungus alone and nematode alone inoculated pots showed 1.86 g and 5.82 g shoot weights respectively. Maximum (9.76 g) shoot weight was recorded in un-inoculated control. Similar trends were also recorded with fresh root weights. The root weight was significantly reduced (0.20 g) in the treatment where nematode preceded fungus followed by simultaneous inoculations by fungus and nematode (0.24 g) and in fungus preceded nematode (0.28 g). All the treatments significantly reduced the root weights (fresh) when compared to control (0.92 g).

On dry weight basis minimum shoot weight (0.26 g) was recorded in plants inoculated with nematode first and fungus a week after followed by simultaneous inoculations of nematode and fungus (0.28 g). Significant reduction in dry shoot weights as compared to control (2.25 g) was recorded in all the treatments. Similar trend was also noted in dry root weights. The root weights (dry) were significantly reduced in all the treatment as compared to control. Minimum root weight was recorded with nematode first and fungus a week after (0.05 g) followed by concomitant inoculations (0.10 g) and fungus first nematode a week after (0.10 g). Nematode alone and fungus alone inoculated pots showed 0.11 g and 0.12 g root weights respectively. Maximum root weight on dry weight basis was recorded with control (0.17 g).

Significantly reduced root length (6.93cm) was recorded with the treatment where *M. incognita* was inoculated prior to *F. oxsysporum* a followed by concomitant inoculations by both the organisms (8.30 cm). Both the organisms inoculated either singly or in combinations significantly reduced root length of tomato plant when compared to un-inoculated control (12.67 cm). Minimum number of galls (27) was recorded with fungus inoculated first and nematode a week after followed by concomitant inoculations (35.67) and nematode first and fungus one week after (44.33). Maximum galling was noted in nematode alone (45.67) against no galling in control and fungus alone.

The data presented in table 1 revealed that maximum number of egg masses (31.33) were noted in nematode alone inoculations followed by nematode inoculated first and fungus a week after (28.67). Simultaneous inoculation of nematode and fungus recorded 23.67 egg masses where as 24.67 egg masses/gall were observed in fungus inoculated first and nematode a week after.Root knot disease caused by *Meloidogyne incognita* and Fusarial wilt by *Fusarium oxysporum* f.sp. *lycopersici* are

individually important diseases leading to extensive yield losses in tomato. These pathogens when interacting with each other can cause high yield loss due to disease complex and may also lead to breaking of resistance (Ansari *et al.,* 2012). This breaking of resistance by *M. incognita* plays a major role in the etiology of the fusarial wilt in tomato posing a serious problem, particularly in the production of resistant cultivars.

Studies were carried out under pot conditions to determine the effect cohabitation of *Meloidogyne incognita* and *F. oxysporum* on disease development and growth parameters in tomato. The results indicated that plant growth was adversely affected in all the cases where plant was inoculated with *M. inocgnita* and *R. Solani* when compared with uninoculated control. Generally, the treatments receiving the nematode inoculation prior to fungus resulted in deritalization of plant completely.

When fungus inoculation was done seven days prior to nematode inoculation, it showed maximum synergistic' effect followed by treatment where both the pathogens were inoculated simultaneously. Sharma and McDonald (1990) also reported that the presence of *Meloidogyne spp* aggravated the disease situation by *F. oxysporium* f.sp. *ciceri* on chickpea. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression. (Malhotra *et al.,* 2011) In a sequential etiology, one pathogen of the disease complex infects the host before the invasion by the other pathogen and brings about certain histophysiological and biochemical alterations within the host, rendering it more suitable substratum for establishment and growth (Anwar and Khan, 2002).

Although, each pathogen was able to reduce the plant growth, the combine infection of nematode and fungus resulted in synerqlstic effect" Haseeb *et al*., 2005 and Ravishankar and Singh, 2008, observed that inoculation of *M. incognita* 15 days prior to *R. solani* significantly reduced all the plant growth parameters as compared to inoculation of *R. solani* 15 days prior to *M. incognita* in *vigna mungo*. Similar effect on suppression of plant growth of tomato than alone treatment was also recorded by (Sarnutniravalf & Sivakumar, 2008).

The host infestation by *M. Incognita* as represented by root knot index was maximum in plants in inoculated nematode seven days prior to fungus followed by nematode and fungus simultaneously, and fungus seven days prior to nematode, nematode alone inoculations showed maximum galling, number of galls and egg masses in roots were found minimum in plants inoculated with fungus first and nematode a week after. This reduction might be due to reduced root system, thus nematode faced competition for food.

In addition to fungal disruption of nematode feeding sites, plants affected by disease complexes may be more prone to early senescence and death which in turn, might prevent nematode from completing its life cycle leading to reduced reproduction. Similar type of reduced galling and population density in presence of *F. oxysporum* was also reported by Roy and Mukhopadhya (2004). Visually highest root infection by *F. oxysporum* was observed in nematode fungus simultaneously inoculated plants followed by nematode seven days prior to fungus, seven days prior to nematode and fungus alone inoculated plants, respectively. There was a significant Increase In percent disease incidence when *M. Incognita* inoculation preceded the fungal inoculation. The result, corroborate with the research findings of Bhagwati *et al.*, (2007).

Table.1 Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* on plant growth parameters

S. No.	Treatments	Plant Height (cm)	Root Length (cm)	Fresh weight (g)		Dry weight (g)		No of Galls/ plant	No of egg Mass/ plant
				Shoot	Root	Shoot	Root		
1	Control	56.23	12.67	9.76	0.92	2.25	0.17	0	0
2	N	43.77	10.90	5.82	0.67	0.56	0.11	45.67	31.33
3	F	43.83	11.50	1.86	0.43	0.81	0.12	0.00	0.00
4	N + F	29.97	8.30	0.75	0.24	0.28	0.10	35.67	23.67
5	N1 + F2	28.07	6.93	0.66	0.20	0.26	0.05	44.33	28.67
6	F1 + N2	31.07	10.80	1.40	0.28	0.30	0.10	27.00	24.67
	S.E(m)±	2.497	1.089	0.655	0.063	0.066	0.023	.308	1.92
	CD at 5 %	7.78	3.422	2.04	0.195	0.207	0.069	10.307	5.981

Mean of three replications.
N - Nematode alone N1 +F2 – Nematode inoculated prior to fungus F – Fungus alone F1 + N2 – Fungus inoculated prior to nematode
N+F–concomitant inoculation

The minimum root infection in plants inoculated with *F. oxysporum* alone than the ones where nematode was present together with fungus suggested that delay in entry of fungus was due to absence of predisposing agent for attracting the fungus to galled roots. Similar type of result was reported by Haseeb *et al.*, (2007) on *Pisum sativum*. These observation on nematode fungal interaction suggested that they were due to nematode providing a readymade means of entry in to the host for the fungus. This occurred when root knot nematode caused superficial root injury and enhances fungal access. The results of the present investigation suggested that *M. Incognita* and *F. oxysporum* together caused grater damage to tomato than either of them alone.

Anwar and Khan (2002) observed slight to moderate galling in nematode inoculated prior to fungus in tomato. This difference might be due to the variety used during the study. The reduction in galling and nematode population could be possibly attributed to deleterious effects of metabolites of *F. oxysporum* on the juveniles of root-knot nematode. This is further supported by greater reduction when fungi and nematodes were inoculated simultaneously.

In conclusion the treatments receiving the nematode inoculation prior to fungus resulted in higher reduction and plant growth than the other treatments. When nematode inoculation was done seven days prior to fungal inoculation, it showed maximum synergistic effect followed by treatment where both the pathogens were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression.

The root knot nematode, *M. incognita* not only predisposed the host but also shortened the incubation period for diseases expression.

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