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Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *cucumerinum on* cucumber grown under protected cultivation

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Abstract
Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. c*ucumerinum on* cucumber grown in the polyhouse under protected conditions. The experiment was conducted in pots to evaluate the effect of nematode and fungus individually as well as in various sequential combinations i.e. simultaneous inoculation of nematode and fungus (SIS), nematode one week prior to fungus (1WBF) and nematode one week after fungus (1WAF). The results revealed that nematode inoculation caused significantly more reduction in plant growth parameter, viz., shoot length (SL), root length (RL), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW) and dry root weight (DRW) of cucumber as a comparison to fungus alone, however, combined inoculation of the nematode and fungus was statistically significant with respect to reduction in plant growth parameter over their individual effect. Maximum and significantly more reduction in plant growth parameters viz., SL (125.5cm), RL (32.0cm), FSW (44.0g), FRW (24.3g), SDW (8.5g) and DRW (4.1g) was observed in the treatment where nematode was inoculated one week before fungus as compares to an untreated check having SL (138.2), RL (47.5), FSW (58.5), FRW (37.5), SDW (21.5), and DRW (9.4). Nematode reproduction in terms of formation of galls and egg masses and final nematode population in soil was also significantly suppressed in the treatment where nematode was inoculated one week prior to fungus.

Keywords: *Meloidogyne incognita*, *Fusarium oxysporum* f. sp. *Cucumerinum*, shoot length, root length, fresh shoot weight, fresh root weight, shoot weight, dry root weight.

Introduction
In India, growing of horticultural crops in polyhouses under protected cultivation is becoming very popular among the farmers throughout the country. Large numbers of polyhouses are being erected in Haryana under the ages of the National Horticulture Mission to grow short duration crops. Cucumber (*Cucumis sativus* L.) is grown all over the world due to a good source of vitamins, minerals, fiber and roughages. Though in the polyhouses, crops are grown under protected conditions, yet the crops are not protected even under protected conditions. Polyhouse cultivation involves intensive cultivation of crops, optimum use of fertilizers and frequent use of irrigation, but continuous growing of the same crop with high day temperature and relative humidity within the greenhouse, polyhouse and low tunnel along with poor plant hygienic conditions inside and outside the greenhouse increase problem of soil borne pests and diseases including plant parasitic nematodes (Minuto *et al*. 2006) [6] which results in the availability of ideal conditions for the growth and multiplication of these pests.

Plant-parasitic nematodes are recognized as major agricultural pathogens and are known to attack plants and cause crop losses throughout the world. Root-knot nematode is the most damaging plant-parasitic nematode (Barker, 1985) [1]. Under polyhouse cultivation crops, are attacked by a number of pests and diseases including nematodes which interfere with the successful cultivation under protected conditions. Among the nematodes, root-knot nematode (*Meloidogyne* spp.) is the most damaging under polyhouse conditions, parasitizing almost all the polyhouses crops. The damage becomes very severe in association with fungi. Though, yield loss due to this nematode is difficult to predict, approximate yield loss due to this nematode has been predicted by many authors in various crops. Another important biotic stress to which the crop exposed is the fungus, *Fusarium oxysporum* f. sp. *cucumerinum.*

Among the vegetable crops grown in the polyhouses in Haryana, cultivation of cucumber is preferred over capsicum and tomato because the farmer gets a good price even in the local market throughout the year. Goel *et al*. (2013) [4] reported the incidence of root-knot nematode

(*Meloidogyne incognita)* in capsicum, cucumber and tomato under protected cultivation in six districts of Haryana with frequency of occurrence of 37.5, 52.9, 33.3%, respectively and low to high nematodes population, however, rose and lilium was found infested with lower population of *Rotylenchulus, Tylenchorhynchus, Ditylenchus, Tylenchulus* and other plant parasitic nematodes.

Generally, root knot nematode-fungus interaction is considered to be one of the important factors responsible for the crop reduction under field conditions. However, very little work has been done on the nematode-fungus interaction in cucumber polyhouses conditions. This research was proposed to study the intensity of damage caused due to nematode-fungus disease complex in cucumber under protected conditions.

Materials and Methods

Propagation of pure culture of *Meloidogyne incognita* for obtaining egg masses and J_2

Identification of root-knot nematode *M. incognita* was done prior to its propagation in pure culture. For this purpose, galled cucumber roots were collected from the naturally infested polyhouses during random survey and brought to the laboratory. Egg masses were separated in sodium hypochlorite solution after continuous stirring for five minutes, for a detachment of egg masses from the roots. Eggs were collected on 500 mesh sieve after proper washing with water to remove the excess sodium hypochlorite. The contents of 500 mesh sieve were taken in a beaker and placed on Modified Baermann's funnel for 24 hours.

After identification, pure culture was propagated with the egg masses collected from root-knot nematode infected roots. A 40-50 earthen pots were filled with steam sterilized soil and four weeks old brinjal seedlings were raised in that pots and inoculated with *M. incognita* juveniles. After 65 days of inoculation, wilted plants showed heavily galling in the roots. Some plants were selected and brought to the laboratory and the same process was repeated for identification of *M. incognita* females through perineal patterns. Egg masses and juveniles from these plants were used in inoculation for further experimentation during the course of present of the investigations. The culture was periodically sub-cultured for multiplication and purity. The desired number of egg masses collected by sodium hypochlorite method were transferred to double folded tissue paper held on a moulded piece of aluminium wire net placed on petri plate at 28 ± 2^0C temperature. Sufficient amount of water was added to keep the egg masses just submerged. On the next day, water from these petri plates containing second stage juveniles was collected in beakers. The number of juveniles was counted per ml solution with three replications. These freshly hatched juveniles were used for inoculation for further experimentation.

Isolation of fungus from plant material collected during random survey

Infected cucumber roots showing symptoms of the disease were obtained from polyhouses during random survey. The roots were cut into small sections (0.5-1.0 cm), washed thoroughly with tap water, surface sterilized with sodium hypochlorite (5%) solution (Nalco) for 5 minutes, rinsed three times in changes of sterilized distilled water and dried on sterilized filter papers. The sterilized root sections were plated at the rate of five sections/ plate onto potato dextrose agar (PDA) in 9-cm Petri dishes. The Petri dishes were incubated at 27±1 °C. After incubation for 7 days, isolated fungi were subculture on PDA. The pure culture of isolated fungus was maintained on PDA slants and renewed after every ten days. Further microscopic examinations were carried out for to the mycelia and conidia structure using pure culture of *F. oxysporum* f. sp. *cucumerinum* was obtained by using Hyphal Tip Technique. Sample of the obtained colonies were sub cultured by transferring small mycelia from the colony margins. Pure cultures were obtained by sub-culturing three times and slides were prepared and examined microscopically to confirm *Fusarium oxysporum* due to occurrence of typical macro conidia with foot-shaped basal cells, micro conidia borne in false heads only on monophialides and chlamydosporia.

Propagation of pure culture of *F. oxysporum* f. sp. *cucumerinum.*

Pure culture of *F. oxysporum* f. sp. *cucumerinum* isolated from the infested plants during a random survey of polyhouses was maintained on PDA in petriplates at (27±1) °C. In order to mass-produce pure culture of the fungus were grown in sand maize meal medium (700gm sand + maize meal 300gm + 150ml distilled water). The flasks and polypropylene bags were incubated in a BOD (Biological Oxygen Demand) incubator at a temperature of (27±1) °C for 15 days. During incubation, the flasks were shaken three times in a day, to ensure proper growth of the fungal mycelium on the sand maize meal medium.

Interaction of *Meloidogyne incognita* and *F. oxysporum* f. sp. *cucumerinum* infesting cucumber grown in polyhouse.

Interaction studies between root-knot nematode and fungus *Fusarium oxysporum* found associated with polyhouse crops during random survey were conducted in pots on cucumber under protected conditions.

Procedure: Autoclaved soil was infested with root-knot nematode @ 1000 J_2 / kg soil and fungus @ 5 g/ pot as per the treatments. The experiment was conducted in pots (1 kg capacity). Each pot was sown with cucumber seeds @ 5 seeds per pot. J_2 and fungus were inoculated as per treatment.

For this experiment, fungus was grown on potato dextrose broth (Potato 200g, dextrose 20g per 1000 ml distilled water) and usual polyhouse care was given.

Treatments

T1: Nematode alone
T2: Fungus alone
T3: Nematode inoculation 7 days after fungus
T4: Nematode inoculation 7 days prior to fungus
T5: Nematode + fungus inoculation simultaneously
T6: Uninoculated check
Replications: Four
Design: CRD

Observations

- Plant growth parameters (shoot length, fresh and dry shoot and root weight)
- Number of galls per plant
- Number of egg masses per plant
- Number of eggs per egg mass
- Final nematode population per pot
- Per cent disease incidence

During the months of April to June, 2015 at the polyhouse under protected conditions at college of agriculture CCS HAU

Hisar, Haryana, India.

Results

Individual effect of *M. incognita* and *F. oxysporum* on plant growth parameters, nematode reproduction and fungus wilt incidence

Nematode and fungus, when inoculated individually caused significant reduction in the plant growth parameters *viz*., shoot length (SL) (95.3 cm), root length (RL) (28.1 cm), fresh shoot weight (FSW) (26.9 g), fresh root weight (FRW) (6.9 g), dry shoot weight (DSW) (7.36 g) and dry root weight (DRW) (2.50 g) of cucumber compared to untreated uninoculated check (Table 2.1). Maximum number of galls (353) per plant, egg masses (494) per plant, eggs per egg mass (288) and final nematode per 200 cc soil (688) was observed with the inoculation of nematode alone (Table 2.2). In the case of plants inoculated individually with fungus 74 per cent wilt incidence of plants was recorded (Table 2.3).

Combined effect of *F. oxysporum* and *M. incognita* on *Fusarium* wilt incidence

Initial symptoms of chlorosis and wilt incidence were observed at 15th and 30thdays after sowing. In the sequential and concomitant inoculation of nematodes and fungus, wilt incidence was higher (83 %) than with the fungus alone (74 %). Presence of nematode contributed to the early onset of wilt symptoms which resulted in more stunting of plants. Sequential inoculation of nematodes 7 days prior to fungus significantly increased the severity of the wilt incidence to 95.5 per cent followed by the concomitant inoculation of nematode and fungus which showed 83.25 per cent of wilt incidence indicating that nematodes predisposed plants to infection by fungus and aggravate the disease incidence which ultimately leads to disease severity.

Combined effect of *F. oxysporum* and *M. incognita* on plant growth parameters

The results revealed that nematode inoculation caused significantly more reduction in plant growth parameter, *viz*., shoot length (SL), root length (RL), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW) and dry root weight (DRW) of cucumber as a comparison to fungus alone, however, combined inoculation of the nematode and fungus was statistically significant with respect to reduction in plant growth parameter over their individual effect. Maximum and significantly more reduction in plant growth parameters *viz*., SL (79.6cm), RL (19.1 cm), FSW (18.1 g), FRW(3.7 g), SDW(3.99 g) and DRW(1.87 g) was observed in the treatment where nematode was inoculated one week prior to fungus as compared to uninoculated check having SL (164.5), RL (61.6 cm), FSW (69.3 g), FRW (24.2 g), SDW (25.7 g), and DRW (8.50).

Combined effect of *F. oxysporum* and *M. incognita* on the severity of nematode reproduction

Concomitant and sequential nematode and fungus inoculation resulted in a significant reduction of the gall index. Maximum galling was observed when the nematodes were inoculated 7 days prior to the fungus. The gall index was reduced with concomitant and sequential inoculation of fungus 7 days prior to nematodes. Severity of root galling diminished as the *Fusarium* density increased due to rotting of roots. This reduced the reproduction potential of the nematode. Similarly, lower nematode female density in roots and juvenile population in the soil were observed in the sequential inoculation of fungus 7days prior to nematodes followed by the concomitant inoculation of nematodes and fungus.

Table 1.1: Interaction of *Meloidogyne incognita* and *Fusarium oxysporum f. sp. cucumerinum* infesting cucumber grown in polyhouse under protected conditions

Treatments	Plant growth parameter					
	Shoot length (cm)	Root length (cm)	fresh shoot weight (g)	Fresh root weight (g)	dry shoot weight (g)	Dry root weight (g)
T1: Nematode alone	95.4	28.2	26.97	6.93	7.36	2.50
T2: Fungus alone	90.3	25.7	24.43	6.07	6.34	2.80
T3: Nematode inoculation 7 days after fungus	99.8	26.7	23.94	6.66	6.61	2.66
T4: Nematode inoculation 7 days prior to fungus	79.6	19.2	18.14	3.70	3.99	1.87
T5: Nematode + fungus inoculation simultaneously	85.6	22.9	20.90	4.33	4.61	2.12
T6: uninoculated check	164.5	61.6	69.37	24.24	25.74	8.50
CD	6.6	2.0	2.5	2.2	1.93	1.60

Table 1.2: Interaction of *M. incognita* and *F. oxysporum f. sp. cucumerinum* infesting cucumber grown in polyhouse under protected conditions.

Treatments	Nematode Reproduction				Percent wilt incidence
	Number of egg masses per plant	Number of eggs per egg mass	Number of galls per plant	Final nematode population per pot	
T1: Nematode alone	494 (22.2)	288 (17.0)	353 (18.8)	688 (26.2)	6.5 (2.54)
T2: Fungus alone	0 (1.0)	0 (1.0)	0 (1.0)	0 (1.0)	74.5 (8.62)
T3: Nematode inoculation 7 days after fungus	413 (20.3)	282 (16.8)	341 (18.5)	524 (22.9)	74.25 (8.61)
T4: Nematode inoculation 7 days prior to fungus	725 (26.9)	289 (17.0)	553 (23.5)	864 (29.4)	95.5 (9.76)
T5: Nematode + fungus inoculation simultaneously	467 (21.6)	287 (17.0)	314 (17.8)	662 (25.7)	83.25 (9.11)
T6: Uninoculated check	0 (1.0)	0 (1.0)	0 (1.0)	0 (1.0)	0 (0.70)
CD	0.08	0.15	0.10	0.09	2.49

Data in parenthesis are the (√n+1) transformed values for nematode reproduction

Table 1.3 Interaction of *M. incognita* and *F. oxysporum f. sp. cucumerinum* infesting cucumber grown in polyhouse under protected conditions.

Treatments	After 15 days	After 30 days
T1: Nematode alone	0 (4.0)	0 (4.0)
T2: Fungus alone	35 (36.3)	70 (57.2)
T3: Nematode inoculation 7 days after fungus	30 (33.5)	65 (54.0)
T4: Nematode inoculation 7 days prior to fungus	60 (51.1)	85 (67.8)
T5: Nematode + fungus inoculation simultaneously	50 (45.2)	75 (60.5)
T6: Uninoculated check	0 (4.0)	0(4.0)
CD	5.60	6.18

Data in parenthesis are the angular transformed values of respective data

Discussion

Very little work has been done on the interaction of *Meloidogyne incognita* and *Fusarium oxysporum f. sp. cucumerinum* infesting cucumber grown in polyhouse. In the present investigation of interaction of root knot nematode with fungus infesting cucumber resulted reduced nematode population and number of gall per plant along with increased fungal wilt incidence were observed with concomitant inoculation of nematode and fungus followed by sequential pathogens inoculation. The interaction studies in various crops under field conditions revealed that nematodes predisposed the plants to the secondary infection by the fungal pathogens which were in agreement with the present studies under polyhouses condition show ever, the result on heavy galling of roots under polyhouse conditions. Concomitant and sequential inoculation of nematodes and fungus resulted in the aggravation of wilt disease severity in cucumber compared with their individual inoculation (74%) under polyhouses conditions. The results in present are in the agreement with the study conducted by Jonathan and Gajendran (1998) [5] in banana cv. Rasthali, where they observed that the incidence of panama wilt disease was severe with the sequential inoculation of *M. incognita* followed by the fungus, *F. oxysporum* f. sp. *cubense* and concomitant inoculation of the two pathogens under the field conditions. Nematode activities in roots modified roots physiology and morphology (Meena *et al*. 2011) [7] which make the plant more vulnerable to the infection by secondary pathogens. In our study nematode reproduction in terms of formation of galls (553) and egg masses per plant (725) and final nematode population in soil (864 J_2/ 200cc soil) was also significantly suppressed in the treatment where nematode was inoculated one week prior to fungus.

Moreover, toxic metabolites produced by the fungi may also reduce the egg hatching of nematodes and immobilize the second-stage juveniles of them (Fattah and Webster, 1989). These findings were confirmed by the present study where reduced nematode population and gall with an increased wilt incidence were observed with concomitant nematode and fungus inoculation followed by sequential pathogens inoculation. Thus, it was proved from the present study that presence of nematode paves way for the early entry of the fungus into the plants which aggravate the wilt disease severity than the individual inoculation of nematodes and fungus.

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