J. Phytol. Res. 16(2) : 143-146, 2003

STUDIES ON THE INTERACTION BETWEEN *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM f* SP. *CUMINI* INFECTING CUMIN (*CUMINUM CYMINUM L.*)

NIDHI SHARMA and P. C. TRIVEDI
Department of Botany, University of Rajasthan, Jaipur 302004, India.

Meloidogyne incognita enhanced wilting of cumin when inoculated in combination with *Fusarium oxysporum*. When nematode inoculation was done 15 days prior to fungal inoculation, it showed maximum effect (Synergistic), followed by the treatment where both the pathogen were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period for disease expression.

Keywords : *Cumin; Fusarium oxysporum f. sp.; Interaction; Meloidogyne incognita.*

Introduction

Cumin (*Cuminum cyminum L.)* is one of the major spice crop growing extensively under irrigated and rainfed conditions in Rajasthan, India. As nature does not play with pure cultures, plants develop in close associations with many soil organisms especially with nematodes and fungi. Out of the nematode-fungus associations infecting cumin, disease-complex caused by root-knot nematode, *Meloidogyne incognita* and wilt fungi, *Fusarium oxysporum f sp. cumini* occurs most frequently in nature. There have been many reports wherein the root-knot nematode has aggravated the disease syndrome caused by wilt fungi, *Fusarium* as has been reviewed by Franci and Wheeler[1]. However, no study has yet been done on the interaction of *Meloidogyne incognita* and *Fusarium oxysporum f. sp. cumini* on cumin, so the present study was undertaken.

Materials and Method

Fungal culture and inoculation- Fusarium oxysporum was isolated from roots and collar regions of cumin plants. These plants exhibited wilting and root browning symptoms. After purification, it was grown on potato dextrose agar (PDA) for 15 days at 25±2°c in a BOD incubator. Pure culture of *Fusarium* prepared on PDA was further multiplied on sorghum grains. Number of spores per gram was counted with the help of haemocytometer. The inoculum level of *Fusarium* used was 20 gm substrate+fungi / pot.

Nematode culture and inoculation -Root-knot nematode *Meloidogyne incognita* was isolated from the same cumin growing field. Pure culture was multiplied on brinjal plants. Just before the inoculation, the feeder roots of the seedlings (10 days old), were exposed by carefully removing the adhering top layer of the soil. The required quantity of nematode suspension having 1000 freshly hatched juveniles, was poured uniformly all over the exposed roots and it was covered immediately with the top soil. This was followed by light watering of the plants.

Interaction between Meloidogyne incognita and Fusarium oxysporum f. sp. cumini.- The interaction experiment was carried out in pot trials; surface sterilized seeds were sown in 18 x 18 cm pots containing autoclaved soil. Experiment was conducted in the month of December. Six treatments were used in the experimental trials viz.

1. Nematode alone (N)
2. Fungus alone (F)
3. Nematode 15 days prior to Fungus ($N \rightarrow F_{15}$)
4. Fungus 15 days prior to Nematode ($N_{15} \leftarrow F$)
5. Nematode and Fungus simultaneously (N+F)
6. Uninoculated control.

Each treatment was replicated five times. Observations for different parameters were recorded after 90 days of treatments.

Wilt Severity score - Foliar wilt severity was recorded by classifying each plant according to the method of Winstead and Kelman[2], 0 = normal; 1 = one leaf partially wilted; 2= two or more leaves wilted; 3 = all except two or three leaves wilted; 4= all leaves

Table 1. Interaction of *M. incognita* and *F. oxysporum* infecting cumin : Effect on plant growth parameters.

Treatments	Pathogen added to the soil	Length (cm)		Fresh wt. (gm)		Dry wt. (gm)	
T_1	N alone	14.67±0.69	10.31±0.41	1.16±0.17	0.59±0.047	0.16±0.026	0.04±0.006
T_2	F alone	16.23±0.57	11.03±0.43	2.04±0.30	0.92±0.040	0.28±0.015	0.08±0.015
T_3	($N \rightarrow F_{15}$)	8.62±0.35	4.04±0.72	0.49±0.093	0.16±0.057	0.05±0.012	0.02±0.006
T_4	($N_{15} \leftarrow F$)	15.01±0.70	10.42±0.52	1.18±0.18	0.60±0.040	0.20±0.012	0.06±0.006
T_5	(N + F)	10.40±0.63	4.26±0.15	0.52±0.09	0.23±0.031	0.05±0.017	0.02±0.006
T_6	Uninoculated control	28.71±1.76	19.18±0.58	3.47±0.27	1.62±0.021	0.43±0.50	0.14±0.020
	CD at 5%	2.80	1.56	0.63	0.12	0.07	0.03

N = Nematode (*Meloidogyne incognita*, F= Fungus (*Fusarium oxysporum f.sp. cumini*), $N \rightarrow F_{15}$ = Nematode 15 days prior to fungus

$NF_{15} \leftarrow F$ = Fungus 15 days prior to nematode. N + F = Nematode and fungus simultaneously

Table 2. Interaction of *M. incognita* and *F. oxysporum* infecting cumin : Effect on host infestation and nematode multiplication.

Treatments	Pathogen added to the soil	No. of galls/ gm root	No. of eggmasses/ gm root	No. of eggs/eggmasses	Root Knot		Wilt	
					Index score (1-5)	Severtiy	Score (1-5)	Symptom index
T_1	N alone	10.91±0.021	19.79±0.15	272.66±11.34	4.2±0.20	84.6±1.33	0.00±0.00	0.00±0.00
T_2	F alone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.2±0.10	44.4±5.02
T_3	($N \rightarrow F_{15}$)	9.53±0.108	18.72±0.35	270.99±8.54	3.4±0.100	78.2±3.39	3.1±0.15	62.7±3.01
T_4	($N_{15} \leftarrow F$)	8.03±0.051	15.76±0.05	224.33±17.57	2.8±0.23	56.4±5.08	2.1±0.05	42.3±5.74
T_5	(N + F)	8.36±0.44	16.85±0.40	233.00±2.51	3.1±0.23	62.1±2.60	2.6±0.30	52.2±3.18
T_6	Uninoculated control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.20	0.00±0.00	0.00±0.00	0.00±0.00
	CD at 5%	0.16	0.71	28.60	0.47	8.53	0.45	11.07

N = Nematode (*Meloidogyne incognita*, F= Fungus (*Fusarium oxysporum f.sp. cumini*), $N \rightarrow F_{15}$ = Nematode 15 days prior to fungus

$N_{15} \leftarrow$ F= Fungus 15 days prior to nematode. N + F = Nematode and fungus simultaneously

wilted; 5= complete death of the plant. Symptom severity for each plant in a treatment was converted to percentage indices as below (Sitaramaiah and Sinha[3])

$$\text{Symptom index} = \frac{\text{Numerical grade accorded to each plant in each treatment}}{\text{Maximum numerical grade}} \times 100$$

Root-knot severity - The intensity of root-knot infestation was recorded according to 1-5 scale (Siitaramaiah and Sinha[3]) and the root-knot severity was calculated using the above formula.

Results and Discussion

Effect on plant growth : The data on the growth parameters of plant are given in Table-1. The results indicated that plant growth was adversely affected in all the cases where plant was inoculated with *Meloidogyne incognita* and *Fusarium oxysporum f. sp. cumini.* The plant height was reduced, roots develop galls and more lateral branching was seen, leaves turned yellow and there was reduction in number of fruits which were of poor quality. Generally the treatments receiving nematode inoculation prior to fungus resulted in higher reduction in plant growth than the other treatments. Although each pathogen was able to reduce the plant growth, the combined infection of nematode and fungus resulted in synergistic effect. The nematode infection 15 days prior to fungus inoculation exposed the host roots favourable to the fungal attack. The simultaneous treatment of both pathogen also expressed a synergistic effect on the hosts. Both the above treatments expressed an early disease severity and also shortened the incubation period for disease expression.

In the present findings, there was a significant reduction in weight of aerial shoot and root in all treatments with joint inoculations of *Fusarium* and *Meloidogyne* (Table-1). *Fusarium* alone and nematode alone treatments showed increase in fresh weight of aerial shoot and root but still were less than the uninoculated check. The plants treated with nematode alone(N) exhibited stunted growth while those inoculated with combination of nematode and fungus also exhibited light yellowing and tip drying of older leaves.

In general, it was observed that the effect of the combined inoculation on plant growth parameters was additive in nature, where inoculations were done simultaneously or the fungus established prior to nematode, wherein the resultant effect on growth parameters was almost equal to sum total of their individual effects. However, in treatments wherein the nematode was established earlier and was followed by the other organism, the resultant effect was more than simple additive.

Effect on wilting of plant : There was a significant increase in cumin wilt when plants were inoculated with 2nd stage juveniles of *M. incognita* in addition to fungus (*F. oxysporum f. sp. cumini).* Among the treatments, maximum wilt score and wilt symptom index were recorded in those plants which received nematodes 15 days prior to fungus followed by simultaneous inoculation of both the pathogens and nematodes 15 days after the fungus. Nematode alone did not produce wilt of cumin. Studies have shown that the wilt expression is greatest when root-knot nematode inoculation preceedes fungal inoculation. Giant cells produced by *Meloidogyne* spp. are known to be a suitable substrate for *F. oxyporum* growth in several systems.

Effect on host infestation and nematode multiplication : Host infestation by nematode as represented by root-knot index was found to be maximum when the nematode occurred individually. The presence of fungus irrespective of time of inoculation affected root galling to varying degrees.

The observations (Table 2) revealed that check plants had healthy normal roots with more primary, secondary and tertiary roots without any galls and decay as compared to others. The plant under fungus alone treatment exhibited ungalled roots.

The nematode multiplication and number of root galls were reduced in presence of fungus. In the inoculation combination where nematode got established prior to fungus viz. N+F and

N_{15} ←F, less number of galls were observed, the reason for which is attributed to reduction in root surface area. However synergistic effect observed in both these treatments may be attributed towards the fact that the host was made more prone to fungal attack. Similar findings were observed by Singh and Goswami[4]. These adverse effects on nematode infestation in presence of fungus was probably mediated by the host[5].

The number of eggmasses per plant and number of eggs per eggmass also followed the same trend. According to powell[5], in combined infection, the fungus component often has a real effect on nematode population and generally population of sedentary forms like *Meloidogyne* spp. is reduced because they remain sedentary at one place and therefore are subjected to influence by changes in the host system as a result of fungal infection. Sethi[6] observed a negative correlation between *Meloidogyne incognita* and *Rhizoctonia solani* while studying the interaction in tomato cv. Moneymaker. Johnson litter[7] and Jorgenson[8] have also recorded such adverse reduction in nematode population in presence of fungus. Ribeiro and Ferraz[9] showed that the population density of nematode in their interaction with fungus is affected by interactive effects of the pathogens, nematodes showing reduced population density in presence of wilt fungus which is in accordance with the present findings.

The presence of nematodes or otherwise have been reported to increase[10] or decrease[11] severity of diseases incited by pathogenic fungi or increase or decrease in nematode infestation either within the tissues due to altered host physiology[7] or outside the tissues[11]. The result of these studies are in agreements with the interrelationship between interacting organisms as postulated by James[11]. According to Morgan-Jones and Rodriguez Kabana division of classification of fungi, *Fusarium oxysporum* as an opportunistic fungus. This fungus rapidly colonizes nematode reproductive structures and destroy females, cysts and eggs particularly those belonging to genera, *Heterodera, Meloidogyne* and *Globodera* where sedentary stages are most vulnerable to the fungal attack. It has been suggested that this vulnerability of eggs, larval and other stages are due to chitinolytic activity of the fungi.

These observations on nematode-fungal interaction suggested that they were due to the nematode providing a ready means of entry into the host for the fungus. Undoubtedly, this occurs when root burrowing nematodes cause superficial root injury and so enhance fungal access and secondary pathogenicity of the root. However, the relationship between two or more parasites on a single host is often more sophisticated than this, especially with endogenous nematodes such as *Meloidogyne spp.*[12-14]

Several other explanations have been put forward to explain the mechanism involved in associate interaction particularly between *Meloidogyne incognita* and *Fusarium oxysporum*. Some of these appear to be indirect and possibly influenced by individual host nematode. Vagrant feeding habits of one species may reduce the number of available feeding sites for the other and there may be other translocated inhibitory factors.

References

1. Francl L J and Wheeler T A 1993, In : *Nematode interactions* (Ed. Khan, M W) Chapman and Hall, London. 79-103.
2. Winstead N N and Kelman 1952, *Phytopathology* **42** 628.
3. Sitaramaiah K and Sinha S K 1984, *Indian J. Nematol.* **14** 1
4. Singh Satyendra and Goswami B K 2001, *Indian J. Nematol.* **31**(2) 139
5. Powell N T 1971, *Phytopathology* **9** 253
6. Sethi C L 1966, *Interrelationship between R. solani and root-knot nematode, M. incognita on tomato.*, Thesis, D.Sc. Imperial College, Univ. of London
7. Johanson A W and Littrell R H 1969, *J Nematol.* **1** 122
8. Jorgenson E C 1970, *J. Nematol.* **2** 393
9. Ribeiro CAG and Ferraz S 1983, *Filopathological Brassleria* **8** 439
10. Powell N T 1963, *Phytopathology* **53** 28
11. James, G L 1966, *Nature* (LOMDOD) **212** 1466
12. Bergeson 1972, *Expl. Parasitol.* **32** 301.
13. Pitcher J R 1974, *The reduction of resistance of tomato to Fusarium wilt by Meloidogyne javanica* (Nematoda) Msc. Thesis, Simon Fraser Univ., Van Couvar 75pp.
14. Garber R H, Jorgenson E C, Smith S and Hyer A H 1979, *J. Nematol.* **11** 133