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INTERACTION BETWEEN ROOT-KNOT NEMATODE AND *FUSARIUM OXYSPORUM* IN FABA BEAN

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Abstract

The effect of sequential and concomitant inoculation with *Fusarium oxysporum* Sclecnt. f.sp. *fabae* Yu and Fong, and *Meloidogyne incognita* (Kofoid & White) Chitwood, at two different levels of nematode infestation on two cultivars of faba bean (*Vicia faba* L.) was studied. Synergistic interaction occurred between the pathogens both in concomitant and sequential inoculations, resulting in a significant reduction in plant growth.

The wilt disease development was significantly higher when nematode is followed by the pathogenic fungus as well as in concomitant inoculations with both pathogen, when compared with the reverse sequence of nematode and fungus.

INTRODUCTION

There is no doubt that more damaging occurs to plants as a result of the combined effects of wilt-inducing fungi and plant-parasitic nematodes. The combination of nematode and fungus often results in a synergistic interaction wherein the crop loss is greater than that of the additive effect of the two pathogens (Francl & Wheeler, 1993). Such an interaction between root-knot nematode and *Fusarium* spp. is known in many crops (Hollis, 1958; Oteifa & Ragab, 1958; Pitcher, 1978; Morrell & Bloom, 1981; Mangat & Bhatti, 1986; Khan & Nejad, 1991 and Fazal, *et al.*, 1994).

Many investigators confirmed the relationship between the presence of root-knot nematode and the increase in the fungal disease incidence. Minton *et al.* (1985) mentioned that nematicides, in addition to reducing nematode damage, also indirectly reduced Fusarium wilt symptoms. Golden and Van Gundy (1975) reported that galled okra and tomato roots infected with *Meloidogyne incognita* in the field and greenhouse were highly susceptible to infection by *Rhizoctonia solani.*

El-Sherif *et al.* (1988) reported that the nematode (*M. incognita*) enhanced the infection with *F. oxysporum* by 40%, infection with *R. solani* by 60%, and infection with *Verticillium* sp. by 36%. Ibrahim *et al.* (1982) reported that the presence of *M. incognita* enhanced the incidence and severity of wilt on cotton by *F. oxysporum* f.sp. *vasinfectum.*

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The synergistic interaction also occurs between *F. oxysporum* and *M. incognita* on banana, in concomitant and sequential inoculations, and leads to significant reduction in plant growth (Jonathan & Rajendran, 1988).

In the present work, the interaction between the wilt pathogen (*F. oxysporum* f.sp. *fabae*) and the root-knot nematode (*M. incognita*) on faba bean plants was studied.

MATERIALS AND METHODS

1. Inoculum preparation:

Inoculum of *Meloidogyne incognita* was originally obtained from the roots of severely infected olive trees in a newly cultivated area in Minufia governorate. The infected roots of olive were chopped into small pieces (1-2 cm) and blended for 45 seconds in a Waring blender with enough water to obtain large number of eggs in water suspension. The number of eggs per 1 ml of this suspension was determined microscopically.

Inoculum of *Fusarium oxysporum* f.sp. *fabae* was prepared by using the spore suspension technique as described by Wensley & Mckeen (1962). Spore suspension was obtained by flooding the fungal cultures grown on PDA plates (7 days old) with sterile water and filtering through three layers of cheese-cloth. The spore suspension was adjusted, using a haemocytometer, to a concentration of 2×10^6 conidia/ml.

2. Interaction between *F. oxysporum* and *M. incognita:*

Plastic pots (15 cm in diam.) filled with steamed sandy loam soil were planted with Giza 429 and Giza 461 faba bean cultivars. Soil infestation with *M. incognita* eggs at two inoculation levels (1000 and 2000 egg/pot) was made one week after germination. Each inoculation level was represented by a set of six pots, in addition to another set free of nematodes serving as a control.

Three weeks after germination, 3 pots of each of the aforementioned sets were inoculated with *F. oxysporum* f.sp. *fabae* spore suspension (50 ml./pot, 2×10^6 spores/ml).

Plant growth parameters (shoots and roots), numbers of galls, egg-masses/gm root system, and number of the second stage juveniles in 250 gram were determined. The experiment was designed in a split plot design. Data were recorded 60 days after nematode inoculation.

3. Study of the interaction under sequential and concomitant inoculation methods:

This experiment was conducted using the faba bean cultivar Giza 461. Nine plastic pots 15 cm diameter, were filled with steamed sandy loam soil and planted with the faba bean cultivars. One week after germination, six pots were infested with *F. oxysporum* alone at the rate of 50 ml each containing 2 x 10^6 spores/pot, while the other 3 pots were inoculated with both pathogens at the same time (*F. oxysporum* and *M. incognita*) at the level of 1000 eggs/pot of *M. incognita* and 10^8 spores of the wilt fungus. Two weeks later, the *Fusarium* infested pots were infested with the nematode at two inoculation levels (1000 and 2000 eggs/pot), 3 pots for each inoculum level.

All pots were arranged on greenhouse bench in a split plot design and all normal cultural practices were conducted as needed. Sixty days after nematode inoculation vascular discoloration was determined following Guber *et al.* (1979) index: 0-5 : 0 = no discoloration and 5 = completely discolored or dead plants and shoot and roots weight and length were recorded.

Parameters related to nematode reproduction (number of galls and egg-masses/ one gram roots), and number of second stage juveniles in 250 grams soil were examined and recorded.

RESULTS AND DISCUSSION

Data in Table (1) show that broad bean plant growth is negatively affected by the infection with either *Meloidogyne incognita* or *Fusarium oxysporum*, however, to different degrees. The combination of both results in, however, greater reductions in the growth parameters tested. The two varieties G461 and G429 behaved differently as to the extent of response to infection. *Fusarium* infection resulted in reduction of shoot and root lengths and weights in the range of 12.9 - 24.7% for length and 12.5 - 36% for weights depending on the variety. Giza 429 appears to be more sensitive to infection compared to Giza 461.

With nematode alone, the same parameters are affected, however, the magnitude of growth reduction is proportional to the level of the nematode inoculum as the higher inoculum is more inhibitive. The root length and weight are more negatively affected compared with the shoot.

Considering the combined inoculation with nematode and *Fusarium* with nema-

todes preceding the wilt fungus, the plant growth parameters are more drastically affected than when each pathogen is present singly.

The inhibition effect ranges between 5.0% and 44.8% for root length depending on the variety and level of nematodes and between 11.1 to 64.4 % for the shoot length in Giza 461; and between 24.7 and 38.2 % in Giza 429. The higher the nematode inoculum, the greater the combined effect of both pathogens.

The root and shoot weights are similarly affected following the same trend experienced with shoot and root length and weight. Root wight reduction ranged from 8.3 to 88.1 % and the range for shoot weight reduction extended from 6.1 to 91.8 %.

Table 1. Effect of the interaction with *F. oxysporum* or *M. incognita* alone or in combination with each other on the plant growth of two faba bean cultivars and reduction %.

Treatments	Shoot length cm		Shoot weight g		Root length cm		Root weight g	
	G461	G429	G461	G429	G461	G429	G461	G429
F. oxysporum	34.3 (23.8)	36.0 (24.7)	21.7 (12.5)	15.3 (36.6)	22.2 (14.6)	21.0 (12.9)	12.3 (15.2)	10.7 (29.1)
N + F (N_1) *	26.5 (41.4)	28.0 (41.4)	15.7 (36.7)	2.7 (88.7)	16.3 (57.3)	13.3 (44.8)	9.0 (37.9)	4.3 (71.5)
N + F (N_2) *	16.0 (64.4)	20.7 (36.7)	602 (75.0)	2.0 (91.8)	11.8 (54.6)	10.2 57.7	2.3 (84.1)	1.7 (88.1)
M. incognita (N_1)	37.7 (11.1)	32 (33.1)	23.3 (6.1)	10.3 (56.9)	24.7 (5.0)	20.3 (15.8)	13.3 (8.3)	5.3 (64.9)
M. incognita (N_2)	35.3 21.7	30.0 (38.2)	22.8 (8.1)	8.7 (63.6)	20.2 (22.3)	15.8 (43.4)	11.0 (24.1)	4.2 (72.2)
Control	45.0	47.8	24.8	23.9	26.0	24.1	14.5	15.1
L.S.D. at 5% for:								
Varieties	NS		1.7		1.7		1.2	
Treatments	5.1		2.9		2.9		2	
Varieties x Treatments	NS		4.1		NS		2.8	

N_1: 1000 eggs/pot; N_2: 2000 eggs/pot * *Fusarium* followed Nematode.
( ) : Reduction %.

Data in Table (2) show the nomatode reproduction expressed in terms of galling, number of egg-masses as well as number of L_2 juveniles in the soil in the presence or absence of *Fusarium*. The vascular discoloration as a result of *Fusarium* infection is also

assessed. The two plant varieties tested expressed some degrees of differences in their response in terms of the above mentioned criteria. Vascular discoloration index resulting from *Fusarium* infection increased from 3.6 with *Fusarium* alone to 3.75 and 5.6 when nematode are present at the two levels (1000 and 2000 eggs/pot) respectively in the variety Giza 461. The respective values are 2.0, 2.75 and 4.75 for Giza 429. Such results indicate that nematodes aggravated the infection and subsequent development of *Fusarium* resulting in higher wilt rating expressed as discoloration. Comparing the two varieties as to the magnitude of increase in wilt through nematodes, it is obvious that G 429 which is moderately susceptible showing vascular discoloration index of 2.0 has increased more than twice (4.75) when nematodes exist at a density of 2000 eggs/pot.

On the other hand, Giza 461, a more wilt -susceptible variety shows an increase in vascular discoloration at a lower rate, *i.e.* 1.67. This indicates that nematode infection predisposes the plant to infection with *F. oxysporum* as reported by Jonathan and Rajendra (1988) on banana and Ibrahim *et al.* on cotton (1982).

Nematodes not only provide a port of entry for the wilt fungus but the plant physiological status is modified so that it becomes less hostile to the fungus and consequently more wilt develops. Similar results have been reported by Powell and Nusbaum (1960) on tobacco for infection with *Phytophthora parasitica* var. *nicotianae*, which grow more luxuriantly in the hyperplastic parenchyma induced by *Meloidogyne incognita acrita*.

For the nematode, it is obvious that galling in Giza 461 increases by increasing the initial inoculum density as 67.7 gall are found per gram of roots with the low inoculum 1000 eggs/pot increasing to 115.3 with 2000 eggs/pot.

The same trend is also found when nematodes and *Fusarium* are present; however, the respective values are somewhat lower. Such a behavior is obvious with respect to number of egg-masses/g. roots and number of juveniles per 250g. soil.

Disease parameters on Giza 429 follow similar trends with some variations as to the numerical values compared with Giza 461. Vascular discoloration increases in the presence of nematodes, however, the variety expresses slightly less susceptibility to *Fusarium*.

With respect to nematode infection and reproduction, galling in G429 is somewhat greater than with Giza 461, with the exception of the treatment N_1 + F, which shows low values for number of egg-masses and L_2 juveniles.

Table 2. The effect of interaction between *F. oxysporum* and *M. incognita* on the fungus infection and nematode reproduction on two faba bean cultivars.

Treatments	Nematode reproduction						Vascular discoloration	
	Galls/g		Egg-masses/g		L_2 in 250 g soil			
	G461	G429	G461	G429	G461	G429	G461	G429
F. oxysporum	-	-	-	-	-	-	3.6	2.0
N + F (N_1) *	54.3	49.0	29.7	4.7	1753.3	173.3	3.75	2.75
N + F (N_2) *	104.3	170.3	56.3	122.7	2380.0	2533.3	5.6	4.75
M. incognita (N_1)	67.7	121.7	44.0	43.3	2560.0	1680.0	-	-
M. incognita (N_2)	115.3	126.7	57.7	47.7	4006.7	7913.3	-	-

L.S.D. 5% for:			
Varieties	5.8	4.4	N.S
Treatments	8.1	6.2	742.6
Varieties x Treatments	11.5	8.8	1050.1

Again, the data relevant to nematodes indicate that the variety Giza 429 is relatively more susceptible to galling compared with Giza 461. In both varieties, the presence of *Fusarium* results in lower numerical values for nematode reproduction parameters, probably through affecting the tissues physiology rendering them less suitable for nematode colonization and reproduction (Jonathan and Rajendran, 1998).

Data in Table (3) show the effect of the relative timing of inoculation with *Fusarium* and nematode on the development of vascular discoloration and nematode reproduction. It is obvious that *Fusarium* alone results in a rate of vascular discoloration of 3.0 which is still unchanged when followed by nematodes at the rate of 1000 nemas/pot. When nematodes level is doubled, the final discoloration rating increased slightly to 3.25. When both are added simultaneously, the vascular discoloration reaches 3.50. On the other hand, when nematodes at the lower level preceded *Fusarium*, the discoloration is 3.75. This obviously indicates that when the plants are infected with nematodes they become more liable to infection with *Fusarium*.

However, nematode reproduction is adversely affected when preceded by *Fusarium* or when both are inoculated simultaneously compared with those found when nematodes exist alone or precede *Fusarium*. This is true for the number of galls/g. root, the number of egg-masses/g. root and the number of L_2 juveniles/250 g soil.

Table 3. Effect of time of inoculation on the nematode reproduction and fungus infection on faba bean cultivar Giza 461.

Treatments	Nematode reproduction			Vascular
	Gall/g. root	Egg-masses / g. root	L_2 in 250 g. soil	discoloration rate
F. oxysporum	-	-	-	3.0
N + F (N_1) *	18.3	5.0	33.3	3.0
N + F (N_2) *	23.3	6.3	80.0	3.25
N + F (N_1) * *	7.3	2.0	13.3	3.5
N + F (N_1) * * *	54.3	29.7	1753.3	3.75
M. incognita (N_1)	67.7	44.0	2560.0	-
L.S.D. (0.05)	3.4	16.5	353.6	-

* : *Fusarium* was added before nematode inoculation (N_1, N_2).

**: Nematode + *Fusarium* were added at the same time (concomitant).

***: Nematode (N_1) was added before *Fusarium* inoculation.

Plant growth parameters are also affected by different inoculation treatments, however, to different extents (Table 4). It is obvious that, whereas *Fusarium* or combinations with nematodes result in reduction in shoot and root growth, compared to the effect of nematode alone. Highest reduction in shoot length is found when nematode preceded *Fusarium* as the shoot reaches 20.5 cm in length and 7.7 g. in weight, compared with 37.3 cm and 25.3 g. respectively with nematode infection alone.

As to the root growth, the least root length is 12.8 cm when N + F are added simultaneously and the weight is 2.3 g. compared to the root growth of 24.5 cm and 13.3 g. when nematodes exist alone. It is obvious that root growth is affected negatively whenever *Fusarium* exist alone or combines with nematode.

Results reported herein confirm the findings of other investigators with respect to the synergestic interaction between nematodes and fungi attacking the root system of many plant species (Powell and Nusbaum, 1960; Pitcher, 1978; Ibrahim, 1982; Jonathan and Rajendran, 1988 and Griggin and Thyr, 1988). Such findings indicate the significance of controlling nematodes as a means for avoiding the aggravation of infection with soil-borne fungi.

Table 4. Effect of time of inoculation on the plant growth of cultivar Giza 461.

Treatments	Shoot		Root	
	Length	Weight	Length	Weight
F. oxysporum	32.0	15.7	17.5	6.3
N + F (N_1) *	30.7	12.3	15.3	4.3
N + F (N_2) *	26.3	10.7	12.7	3.8
N + F (N_1) * *	24.3	9.3	12.3	2.3
N + F (N_1) * * *	20.5	7.7	15.7	2.7
M. incognita (N_1)	37.3	25.3	24.5	13.3
L.S.D. (0.05)	9.9	5.5	5.4	2.5

* : *Fusarium* was added before nematode inoculation (N_1, N_2).

**: Nematode + *Fusarium* were added at the same time (concomitant).

***: Nematode (N_1) was added before *Fusarium* inoculation.

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العلاقة بين فطر فيوزاريوم أوكسيسبورم ونيماتودا تعقد الجذور فى الفول البلدى

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مركز البحوث الزراعية – معهد بحوث أمراض النباتات

تم دراسة العلاقة بين الفطر فيوزاريوم أوكسيسبورم ونيماتودا تعقد الجذور من النوع ميلويدجينى إنكوجنيتا والعوامل المؤثرة على التفاعل بينهما تحت ظروف العدوى الصناعية بالصوبة على نبات الفول البلدى.

وقد وجد أن هناك علاقة تعاونية بين المسببان المرضيان الفطرى والنيماتودى.

عند إضافة العدوى سواء بأسبقية أحدهما للآخر أو بعدوى الأثنين معا فى وقت واحد نتج عنها انخفاض فى نمو النباتات.

وجد كذلك أن تطور مرض الذبول كان أعلى بدرجة معنوية عندما أضيفت النيماتودا أولاً ثم تبعها الفطر وكذلك عند اضافة الأثنين معاً فى وقت واحد مقارنة بأسبقية العدوى بالفطر للنيماتودا.