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Interaction between Certain Root-Rot Fungi and the Root-Knot Nematode, *Meloidogyne incognita* on Sunflower Plants

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Effects of the root-rot fungi: *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium solani* on sunflower plants cv. Myak infected by *Meloidogyne incognita* were studied. The results indicated that combined infection with *M. incognita* plus any of the tested fungi resulted in significant reduction in number of root galls and nematode egg masses. Plant growth reduction and disease severity were greatly evident when *M. incognita* was inoculated simultaneously with *R. solani*. Inoculation with *M. incognita* alone or in combination with three different inoculum levels of *R. solani*, *M. phaseolina* or *F. solani* on sunflower cv. Myak was studied in three greenhouse experiments. Treatments with *M. incognita* in combination with different inoculum levels of the tested fungi caused significant reduction in number of root galls and nematode egg-masses and the dry weight of sunflower plants.

Effect of inoculation time of *M. incognita* applied alone or in combination with *R. solani*, *M. phaseolina* and *F. solani* on sunflower cv. Myak was also studied. Treatments with any of the tested fungi either at the same time or one week before *M. incognita* inoculation resulted in significant reduction in number of root galls and nematode egg-masses. Treatments with *M. incognita* alone or in combination with any of the examined fungi caused significant reduction in the dry weight of sunflower plants.

Keywords: *Fusarium solani*, *Macrophomina phaseolina*, *Meloidogyne incognita*, *Rhizoctonia solani*, root-rot and sunflower plants.

Oil field crops represent a great economic importance in the Egyptian agriculture and industry. Sunflower crop plays a major role in the diet of many people in Egypt and other countries. Previous investigations showed that sunflower plants have been attacked by many phytoparasitic nematodes and pathogenic fungi (Shohla, 1980; Sharma, 1990 and Ahmed *et al.*, 1994). Several root pathogenic fungi attack sunflower plants during the growing season inducing diseases such as damping-off, root rot, charcoal rot and wilt. Previous studies showed that the root-rot fungi: *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum*, *Macrophomina phaseolina*, *Phytophthora nicotianae* var *parasitica*, *Pythium irregulare*, *Sclerotinia sclerotiorum* and *S. rolfsii* were pathogenic to germinating seeds and seedlings of sunflower (Sadashivaiah *et al.*, 1986; Zazzerini and Tosi, 1987; Ahmed *et al.*, 1994

and EL-Komy, 2001). Importance of the plant parasitic nematodes in root disease complex has received much attention. Many reports have shown that infection with the root-knot nematodes (*Meloidogyne* spp.) and some root pathogenic fungi may result in greater damage on the host plant than either pathogen acting alone (France and Abawi, 1994; Mahgoub, 1996; Mohamed *et al.*, 1990; Shahda and EL-Saedy 1990; Srivastava and Singh, 1991 and Khan and Hossini-Nejad, 1991).

The present research was carried out to study effects of the root-rot fungi: *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* alone or in combination with *Meloidogyne incognita* on sunflower plants cv. Myak.

Materials and Methods

Nematode inoculum preparation:

Females and egg-masses of *Meloidogyne incognita* were isolated from infected eggplant roots. Cultures of this nematode species were established from single egg-masses of adult females previously identified by the morphological characteristics of the female perineal patterns (Taylor and Sasser, 1978) and reared on Rutgers tomato plants in a greenhouse. The root-knot nematode eggs were extracted from infected tomato roots using sodium hypochlorite (NaOCl) solution as described by Hussey and Barker (1973).

Source of fungal pathogens:

Fungal pathogens used in this study, *i.e. Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani,* were obtained from the culture collection of the Department of Plant Pathology, Faculty of Agriculture, Alexandria University.

Effect of R. solani, F. solani and M. phaseolina alone or in combination with Meloidogyne incognita on sunflower plants:

A greenhouse experiment was conducted to determine the effect of *R. solani, F. solani* or *M. phaseolina* alone or in combination with *M. incognita* on sunflower cv. Myak. Sunflower seeds were sown in 15 cm diam clay pots filled with autoclaved soil mixture of 1: 1 (v: v) sand and clay. One week after emergence, seedlings were thinned to two per pot. About 35 grams of barely grains infested with either *R. solani, F. solani* or *M. phaseolina* were used as a fungal inoculum/pot. Treated pots were inoculated with 5000 nematode eggs/pot at the same time of fungal inoculation. Uninoculated pots were served as a check. Treatments were replicated 4 times. Pots were arranged in a randomized complete block design. The experiment was terminated 45 days after nematode inoculation. Number of root galls and egg-masses and the dry weights of shoot and root systems were determined.

Effect of different inoculum levels of R. solani, F. solani and M. phaseolina alone or in combination with Meloidogyne incognita on sunflower plants:

Three greenhouse experiments were carried out to study the effect of three different inoculum levels of *F. solani, M. phaseolina* or *R. solani,* alone or in combination with *M. incognita* on sunflower cv. Myak. Sunflower seeds were sown in 15 cm diam clay pots containing autoclaved sandy clay soil. A total of 36 pots

were used for each fungus. Seven days after emergence, seedlings were thinned to two/pot. Three different fungal inoculum levels of each fungus were used as follows; 20, 35 and 50 g of infested barley grains/pot.

Treatments included fungal inoculation and fungus plus 5000 nematode eggs/pot at the same time of fungal inoculation. Uninoculated pots served as a check. Treatments were replicated four times. Pots were arranged in a randomized complete block design. Plants were harvested 45 days after fungal and/or nematode inoculation. The number of root galls and nematode egg-masses and dry weight of shoot and root systems were assessed.

Effect of inoculation time of Meloidogyne incognita alone or in combination with R. solani, F. solani or M. phaseolina on sunflower plants:

Three greenhouse experiments were conducted to determine the effect of time of inoculation with *M. incognita* alone or in combination with *F. solani, M. phaseolina* or *R. solani* on growth of sunflower cv. Myak. Sunflower seeds were sown in 15 cm diam clay pots filled with autoclaved sandy clay soil 1:1 (v:v). A total of 20 pots were used for each fungus in a separate experiment. One week after emergence, seedlings were thinned to two per pot. Thirty grams of infected barley grains were used as a fungal inoculum from each fungus per pot. Pots were inoculated with 5000 nematode eggs per pot either 1 week prior or 1 week later or at the same time of fungal inoculation. Uninoculated pots served as a check. Treatments were replicated 4 times. Pots were arranged in a randomized complete block design. The experiments were terminated 45 days after nematode inoculation. Number of root galls and egg-masses and dry weight of shoot and root systems were determined.

Statistical Analysis:

Analysis of variance using SAS software (SAS Institute, 1988) was carried out on plant growth measurements. Data of number of nematode galls and egg-masses were transformed to $\sqrt{x+1}$ before statistical analysis. Treatment means were compared with revised LSD test at the 5% level of probability.

Results

Effects of infection with Rhizoctonia solani, Fusarium solani or Macrophomina phaseolina alone or in combination with Meloidogyne incognita on sunflower plants:

Data in Table (1) show that treatment with *M. incognita* induced great number of root galls and nematode egg-masses on sunflower plants cv. Myak. However, inoculation with *M. incognita* in combination with the tested fungi resulted in significant reduction in number of root galls and nematode egg-masses in comparison with those of the nematode treatment. Treatments with *M. incognita* alone or in combination with the tested fungi showed insignificant differences in shoot or root dry weights in comparison with those of control treatment.

Table 1. Effect of infection with *Rhizoctonia solani*, *Fusarium solani* or *Macrophomina phaseolina* on dry weight and disease severity of sunflower cv. Myak infected by *Meloidogyne incognita*

Treatment	Dry weight (g)		No. of root galls & egg-masses/ plant	
	Shoot	Root	galls	egg masses
Control	2.65 a	1.33 a	-	-
M. incognita	2.73 a	1.21 a	1054 a	1005.8 a
R. solani	2.57 a	0.86 a	-	-
MI+ *R. solani*	2.12 a	1.34 a	674 b	638.8 b
F. solani	3.10 a	0.87 a	-	-
MI+ *F. solani*	3.29 a	1.31 a	574.8 bc	534.5 bc
M. phaseolina	3.11 a	1.24 a	-	-
MI+ *M. phaseolina*	3.17 a	0.85 a	420.5 c	402.8 c

Data are averages of 4 replicates. Values in the column followed by the same letter(s) are not significantly different ($P = 0.05$).

Effects of infection with three inoculum levels of Rhizoctonia solani alone or in combination with Meloidogyne incognita on sunflower plants:

Data in Table (2) show that infection with *M. incognita* induced great number of galls and egg-masses on roots of sunflower cv. Myak. However, infection with *M. incognita* in combination with the different inoculum levels of *R. solani* showed significant decrease in number of root galls and nematode egg-masses developed on sunflower plants in comparison with those of the nematode treatment. Treatments of *M. incognita* alone or in combination with the different inoculum levels of *R. solani* induced significant reduction in shoot and root dry weights in comparison with those of the control treatment.

Table 2. Effect of infection with three inoculum levels of *Rhizoctonia solani* on dry weight and disease severity of sunflower cv. Myak infected by *Meloidogyne incognita* (MI)

Treatment *	Dry weight (g)		No. of root galls & egg-masses/ plant	
	Shoot	Root	galls	egg masses
Control	10.46 a	2.75 a	-	-
M. incognita	4.76 b	1.13 cd	1644.0 a	1599.3 a
R. solani L1	3.93 b	1.43 bcd	-	-
MI+*R. solani* L1	5.26 b	1.67 b	1253.0 b	1092.5 b
R. solani L2	4.39 b	1.11 cd	-	-
MI+*R. solani* L2	4.18 b	1.50 bc	961.8 c	935.5 c
R. solani L3	3.90 b	0.97 d	-	-
MI+*R. solani* L3	3.89 b	1.07 cd	907.8 c	893.0 c

* L1= Low level, 20g/pot. L2 = Medium level, 35g/pot. L3= High level, 50g/pot.
Data are averages of 4 replicates. Values in the column followed by the same letter(s) are not significantly different ($P = 0.05$).

Effects of infection with three inoculum levels of Fusarium solani alone or in combination with Meloidogyne incognita on sunflower plants:

Data in Table (3) show that infection with *M. incognita* alone induced great number of root galls and egg masses on sunflower plants cv. Myak. Inoculation with different levels of *F. solani* plus *M. incognita* resulted in significant reduction in number of root galls and nematode egg-masses developed on sunflower plants in comparison with those of the nematode treatment.

Inoculation with *F. solani* at the low (20g/pot) or at the high (50g/pot) levels caused a significant increase in root dry weight in comparison with those of the nematode treatment (Table 3).

Table 3. Effect of infection with three inoculum levels of *Fusarium solani* on dry weight and disease severity of sunflower cv. Myak infected by *Meloidogyne incognita* (MI)

Treatment *	Dry weight (g)		No. of root galls & egg-masses/ plant	
	Shoot	Root	galls	egg masses
Control	4.79 ab	2.13 a	-	-
M. incognita	2.89 c	0.82 c	963.6 a	915.6 a
F. solani L1	5.33 a	1.63 ab	-	-
MI+*F. solani* L1	3.45 bc	1.28 bc	693.1 b	649.0 b
F. solani L2	4.12 abc	1.35 abc	-	-
MI+*F. solani* L2	3.72 abc	0.80 c	481.5 c	443.4 c
F. solani L3	3.12 bc	1.18 bc	-	-
MI+*F. solani* L3	4.78 ab	0.69 c	471.9 c	433.5 c

* L1= Low level, 20g/pot. L2 = Medium level, 35g/pot. L3= High level, 50g/pot.
Data are averages of 4 replicates. Values in the column followed by the same letter(s) are not significantly different ($P = 0.05$).

Effects of infection with three inoculum levels of Macrophomina phaseolina alone or in combination with Meloidogyne incognita on sunflower plants:

Data in Table (4) show that treatment with *M. incognita* induced great number of root galls and egg-masses on infected sunflower plants. Inoculation with *M. phaseolina* plus *M. incognita* caused significant reduction in number of root galls and egg-masses developed on sunflower plants in comparison with those of the nematode treatment. Inoculation with *M. incognita* alone or in combination with the different inoculum levels of *M. phaseolina* induced significant reduction in shoot dry weight in comparison with those of control. Inoculation with *M. incognita* alone or in combination with the medium inoculum level (35g/pot) or the high (50g/pot) levels of the fungus showed significant reduction in root dry weight in comparison with those of control.

Table 4. Effect of infection with three inoculum levels of *Macrophomina phaseolina* on dry weight and disease severity of sunflower cv. Myak infected by *Meloidogyne incognita* (MI)

Treatment *	Dry weight (g)		No. of root galls & egg-masses/ plant	
	Shoot	Root	galls	egg masses
Control	13.17 a	1.38 a	-	-
M. incognita	2.43 b	0.63 b	1096.1 a	1016.9 a
M. phaseolina L1	2.40 b	1.09 ab	-	-
MI+*M. phaseolina* L1	2.99 b	0.99 ab	591.6 b	559.0 b
M. phaseolina L2	2.22 b	0.97 ab	-	-
MI+*M. phaseolina* L2	2.78 b	0.77 b	470.8 c	447.5 c
M. phaseolina L3	2.31 b	0.69 b	-	-
MI+*M. phaseolina* L3	2.51 b	0.59 b	495.0 c	445.6 c

* L1= Low level, 20g/pot. L2 = Medium level, 35g/pot. L3= High level, 50g/pot.
Data are averages of 4 replicates. Values in the column followed by the same letter(s) are not significantly different ($P = 0.05$).

Effect of inoculation time of Meloidogyne incognita alone or in combination with Rhizoctonia solani on sunflower plants:

Data in Table (5) show that inoculation with *M. incognita* resulted in developing great number of root galls and egg-masses on the infected sunflower plants. Treatments of *M. incognita* alone or in combination with *R. solani* one week later showed more root galls and egg-masses than those of the other treatments. Treatments of *R. solani* when applied either at the same time or one week before nematode inoculation resulted in significant reduction in number of root galls and nematode egg-masses in comparison with those of the nematode treatment. Treatment with *R. solani* before or after nematode inoculation showed a significant reduction in root dry weight in comparison with those of the control.

Table 5. Effect of inoculation time of *Meloidogyne incognita* (MI) alone or in combination with *Rhizoctonia solani* on dry weight and disease severity of sunflower cv. Myak

Treatment	Dry weight (g)		No. of root galls & egg-masses/ plant	
	Shoot	Root	galls	egg masses
Control	7.67 a	1.41 a	-	-
M. incognita	4.50 a	1.17 ab	1943.0 a	1850.8 a
MI+*R. solani* 1 week later	5.30 a	0.94 b	1853.0 a	1785.0 a
R. solani +MI 1 week later	4.25 a	0.88 b	945.0 b	901.8 b
MI+*R. solani* at same time	5.17 a	1.23 ab	1246.5 b	1205.8 b

Data are averages of 4 replicates. Values in the column followed by the same letter(s) are not significantly different ($P = 0.05$).

Effects of inoculation time of Meloidogyne incognita alone or in combination with Fusarium solani on sunflower plants:

Data in Table (6) indicate that infection with *M. incognita* alone resulted in developing great number of root galls and egg-masses on sunflower plants cv. Myak. Treatments with *F. solani* either one week before or at the same time of nematode inoculation showed significant reduction in number of root galls and nematode egg-masses. Treatment with *F. solani* one week after *M. incognita* inoculation showed non-significant differences in root galls and nematode egg-masses in comparison with those of the nematode treatment. The different treatments of the fungus in combination with the nematode caused significant reduction in root dry weight in comparison with those of control (Table 6).

Table 6. Effect of inoculation time of *Meloidogyne incognita* (MI) alone or in combination with *Fusarium solani* on dry weight and disease severity of sunflower cv. Myak

Treatment	Dry weight (g)		No. of root galls & egg-masses/ plant	
	Shoot	Root	galls	egg masses
Control	4.61 a	1.68 a	-	-
M. incognita	4.36 a	1.24 ab	996.3 a	977.3 a
MI+*F. solani* 1 week later	3.54 a	1.01 b	1022.8 a	974.3 a
F. solani + MI 1 week later	4.04 a	0.81 b	479.5 c	429.5 c
MI+*F. solani* at same time	3.65 a	0.92 b	771.3 b	747.3 b

Data are averages of 4 replicates. Values in the column followed by the same letter(s) are not significantly different ($P = 0.05$).

Effects of inoculation time of Meloidogyne incognita alone or in combination with Macrophomina phaseolina on sunflower plants:

Data in Table (7) show that infection with *M. incognita* alone induced great number of root galls and egg-masses on sunflower plants cv. Myak. Treatments with *M. phaseolina* either at the same time or one week before nematode inoculation resulted in significant reduction in number of root galls and nematode egg-masses. Treatment with *M. incognita* one week before fungus inoculation showed insignificant reduction in number of root galls and nematode egg-masses in comparison with those of the nematode treatment alone. The different treatments with *M. phaseolina* in combination with the nematode caused significant reduction in root dry weight in comparison with those of check treatment (Table 7).

Table 7. E fect of inoculation time of *Meloidogyne incognita* (MI) alone or in cc mbination with *Macrophomina phaseolina* on dry weight and di ease severity of sunflower cv. Myak

Treatment	Dry weight (g)		No. of root galls & egg-masses/ plant	
	Shoot	Root	galls	egg masses
Control	5.41 a	2.44 a	-	-
M. incognit	3.12 b	1.12 b	1084.8 a	1026.3 a
MI+*M. pha* *eolina* 1 week later	2.93 b	0.74 b	968.3 a	937.5 a
M. phaseoli *a* + MI 1 week later	2.69 b	0.78 b	467.5 c	428.8 c
MI+*M. pha* *eolina* at same time	2.47 b	0.74 b	726.5 b	665.3 b

Data are aver ges of 4 replicates. Values in the column followed by the same letter(s) are not significantly (ifferent ($P = 0.05$).

Discussion

The pre nt results revealed that infection with *F. solani*, *R. solani* or *M. phaseolina* plus *M. incognita* induced significant reduction in root galls and nematode e g-masses on sunflower plants. Previous studies by Al-Hazmi (1985) indicated th t infection and reproduction of *M. javanica* were affected when combined ith *M. phaseolina*. The nematode galls and egg-masses were significantly decreased when compared with those of the nematode treatment. It was noticed that the combined infection with the nematode and fungus decreased nematode re roduction and this may be due to production of fungal toxins, adverse effect of the fungus on the nematode penetration and/or fungal invasion of giant cells which disrupts nematode feeding. Ayvar-Serna *et al.* (1990) reported that interaction b tween *F. oxysporum* f. sp. *lycopersici* and *M. incognita* on tomato plants reduce d galling and nematode reproduction. Also, Mehta *et al.* (1990) showed that neither *Rhizoctonia solani* nor *Macrophomina phaseolina* had any effect on growth of F ench bean (*Phaseolus vulgaris*) plants, but both fungi affected *M. javanica* rep oduction. Also, *R. solani* reduced number of galls and egg-masses per plant when a ded immediately before nematode inoculation.

The obta ned data showed that infection with *M. incognita* plus different inoculum lev els of *R. solani*, *M. phaseolina* or *F. solani* significantly decreased number of ro t galls and nematode egg-masses developed on sunflower plants. Also, increasing fu gal inoculum level induced great reduction in nematode infection and reproduction. These results are in agreement with those of Siddiqui and Husain (1991) who ndicated that disease severity on chickpea plants was increased with increasing *M. incognita* and *M. phaseolina* inocula.

In the pr ent study, increase of the inoculum level of *M. phaseolina* induced a marked dec ease in root galls and nematode reproduction of *M. incognita*.

Also, results showed that treatments with *F. solani*, *R. solani* or *M. phaseolina* either at the same time or one week before *M. incognita* inoculation resulted in significant reduction in number of root galls, nematode egg-masses and growth parameters of sunflower plants. These results are in agreement with those of Zaidi and Tiyagi (1989) and Anwar and Verma (1993).

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التداخل بين بعض فطريات أعفان الجذور ونيماتودا تعقد الجذور *Meloidogyne incognita* على نباتات عباد الشمس

أسماء عبد الحميد مقبل ، إبراهيم خيري عتريس إبراهيم ،
محمد ربيع عبد المعز شحاته ومحمد أنور محمد الصعيدي
قسم امراض النبات – كلية الزراعة – جامعة الاسكندرية .

تم دراسة تاثيرالعدوي المشتركة بنيماتودا تعقد الجذور *Meloidogyne incognita*وبعض فطريات اعفان الجذور *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* على نباتات عباد الشمس صنف ميك , واوضحت النتائج ان العدوى المشتركة تسبب نقص معنوى فى أعداد العقد الجذرية وكتل بيض النيماتودا المتكونة على النباتات المعاملة بكل من النيماتودا واي من هذه الفطريات المختبرة مقارنة بمعاملة النيماتودا فقط .

كما تم دراسة تاثير كل من الفطريات *M. phaseolina, F. solani, R. solani* النامية على حبوب شعير و بثلاثة مستويات لقاح مختلفة (20، 35، 50جم/أصيص) سواء منفردة او بالاشتراك مع نيماتودا تعقد الجذور *M. incognita* على نباتات عباد الشمس صنف ميك . حيث اوضحت النتائج ان المعاملة بأي مستوى من مستويات اللقاح لاي من الفطريات السابقة ادى الى نقص معنوى فى عدد العقد الجذرية وكتل بيض النيماتودا المتكونة على النباتات المعاملة بالنيماتودا واي من مستوى الفطريات السابقة مقارنة بالمعاملة بالنيماتودا فقط .

كذلك تم أجراء ثلاثة تجارب لدراسة تاثير وقت اضافة العدوى بنيماتودا تعقد الجذور *M. incognita* و كل من الفطريات *M. phaseolina F. solani, R. solani* على نباتات عباد الشمس صنف ميك, حيث تمت إضافة لقاح الفطر مع لقاح النيماتودا فى نفس الوقت أو قبل ذلك أو بعد ذلك بمدة أسبوع. وقد أظهرت النتائج أن العدوى بأى من هذه الفطريات مع النيماتودا فى نفس الوقت أو قبل عدوى النيماتودا بأسبوع نتج عنه نقص معنوى فى أعداد العقد الجذرية وكتل البيض مقارنة بالعدوى المنفردة بالنيماتودا. كذلك ظهر ان العدوى بكل من *F. solani, M. phaseolina, R. solani* مع النيماتودا على أختلاف أوقات أضافتها ادت الي حدوث نقص معنوى في الوزن الجاف لنباتات عباد الشمس.