

التفاعل بين فطري *Rhizoctonia solani* و *Fusarium oxysporum*

ونيماتودا تعقد الجذور *Meloidogyne spp.* على نباتات البطاطس

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الملخص العربى :

تم دراسة تأثير كل من الفطرين *R. solani* و *F. oxysporum* ونيماتودا تعقد الجذور *Meloidogyne spp.* منفردين أو مجتمعين على نبات البطاطس صنف نيقولا تحت ظروف الصوبة . وقد أوضحت النتائج حدوث زيادة معنوية لمعدل حدوث الذبول لنباتات البطاطس فى حالة إضافة النيماتودا أو النيماتودا وفطر *R. solani* مع الفطر المسبب للذبول (*F. oxysporum*) مقارنةً بالمعاملة بفطر الذبول فقط . كما أوضحت النتائج أن نسبة الإصابة ومعدل الإصابة للدرنات بمرض القشرة السوداء ومرض تقرح الساق قد زاد بصورة معنوية عند المعاملة بالنيماتودا أو النيماتودا وفطر *R. solani* مع فطر *F. oxysporum* مقارنةً بالمعاملة بفطر *R. solani* فقط . وقد لوحظ حدوث نقص معنوى فى كل صفات النيماتودا تحت الدراسة (عدد الطور اليرقى الثانى فى التربة - عدد كتل البيض - عدد الإناث - عدد الأطوار المختلفة للنيماتودا - معدل التكاثر) عند إضافة كل من الفطرين *R. solani* و *F. oxysporum* للنباتات المعاملة بالنيماتودا مقارنةً بمعاملة النيماتودا فقط . كان لمعاملة نبات البطاطس بالمسببات المرضية مجتمعة تأثير كبير فى حدوث نقص معنوى لكلٍ من وزن الجذور ، وزن المجموع الخضرى ، وزن الدرناات ، وارتفاع النبات مقارنةً بهذه القياسات فى النباتات المعاملة بكل مسبب مرضى على حده .

INTERACTION EFFECT BETWEEN *FUSARIUM OXYSPORUM*, *RHIZOCTONIA SOLANI* AND *MELOIDOGYNE* SPP. ON POTATO PLANTS

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ABSTRACT: *The effect of three pathogenic organisms, Fusarium oxysporum, Rhizoctonia solani and mixed group of Meloidogyne spp. (M. javanica and M. incognita) separately or in a combination on potato plant cv. Nicola was studied under greenhouse conditions. The combined infection with mixed Meloidogyne spp. plus tested fungi resulted in significant reduction in all nematode parameters, No. of J₂ / soil, No. of developmental stages, No. of Females, No. of egg masses and reproduction factor compared with nematode treatment only. Disease severity of Fusarium wilt was greatly increased when mixed Meloidogyne spp. and R. solani were combined with F. oxysporum compared with wilt fungus treatment only. However, significant increase was observed on black scurf, stem canker diseases and infected tuber when mixed Meloidogyne spp. and F. oxysporum were combined with R. solani compared with R. solani treatment only. The interaction between the tested pathogenic organisms was more effective on reduction plant growth parameters reduction than each of this organisms separately.*

Key words: *Fusarium oxysporum, Rhizoctonia solani, Meloidogyne spp., Fusarium wilt, black scurf, stem canker, potato plants and interaction effect.*

INTRODUCTION

Potato, *Solanum tuberosum* L. is considered one of the most important vegetable crops in Egypt as well as many other countries in the world. It plays an important role in Egyptian agricultural economy, not for local consumption but also for exportation.

Potato is heavily attacked by different pathogen causing severe damage by reducing number and size of tubers production, as well as by lowering the marketability of infected tubers.

Fusarium oxysporum is a serious fungus causes vascular wilt, stunting, chlorosis and eventual plant death on many important crops (Kucharek *et al.*, 2002; Norgues *et al.*, 2002 and Daami-Remadi and El-Mahjoub, 2004). *F. oxysporum* caused 10 to 53% losses of potato yield (Thanassoulpoulos and

Kitsos, 1985).

Black scurf of potato caused by *Rhizoctonia solani* is an important disease commonly observed in most potato areas. The disease can greatly affect tuber quality and therefore can severely reduce its market value (Abdessamad and Maria, 2006; Grosch *et al.*, 2005; Peplmjak, 1999 and Banyal, 2002). The fungus *R. solani* affect the plant from the initiation of sprout emergence through the harvest of tuber (Verma, 1996).

Root-knot nematode is one of the major obstacle facing the production of potato and caused considerable damage (Shady, 2001 and Abd-Allah, 1999). It causes loss reaches to U.S \$ 100 million loss / year worldwide (Oka *et al.*, 2000).

Many reports cleared that infection with root-knot nematodes (*Meloidogyne*

spp.) and some root pathogenic fungi may result in greater damage on the host plant than either pathogen acting alone (Mahgoub, 1996). *Fusarium* wilt in tomato increased when *M. incognita* was present in soil (Awad *et al.*, 1997). Interaction of *R. solani* with *M. javanica* resulted a reduction in the number of nematode galls, root necrosis and damage to plant growth was highest when either pathogen acting alone (Walia and Gupta, 1994).

The present research was carried out to study effects of *Fusarium oxysporum*, *Rhizoctonia solani* and mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) separately or in combination on potato plant cv. Nicola.

MATERIALS AND METHODS

This work was carried out under greenhouse conditions at the Experimental Farm, Faculty of Agriculture, Minoufiya University, Shibin El-Kom during 2010-2011 growing seasons.

1. Isolation and identification of the fungi:

Potato plants (tubers and roots) showing diseases symptoms were cut into small pieces and washed thoroughly with tap water to remove any adhering soil particles, surface sterilized with ethyl alcohol 70 % for three minutes then rinsed three times in sterilized water, dried between two sterilized filter paper and aseptically transferred into plates containing potato dextrose agar (PDA) medium amended with 150 ppm streptomycin sulfate to avoid bacterial contamination. Petri dishes were incubated at 22°C for 3-5 days and examined daily for occurrence of mycelial growth.

The isolated fungi were purified using the hyphal tip technique and transferred to slants of PDA medium incubated for five days at 25°C then stored at 5°C for further studies. The growing fungi were

examined microscopically and identified in Agricultural Botany Department, Faculty of Agric, Minoufiya Univ.

2. Preparation of *Meloidogyne* spp. inoculums:

Two months old Night shade (*Solanum nigrum* L.) roots infested with mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) were removed from the pots and gently washed with tap water to remove the adherent soil particles, cut into small pieces and then macerated for two periods of 10 seconds at high speed using a blender. The macerated roots were placed in Duran bottle containing sodium hypochlorite (NaOCl) 0.5% concentration as described by (Hussey and Barker 1973). The solution in Duran bottle was vigorously shaken for three minutes to release the eggs from the egg matrix as NaOCl removes the gelatin matrix of egg masses. The solution was poured through different size sieves (75 and 38 micrometer, respectively). Then eggs were transferred into Baermann trays with soft tissue paper to allow egg hatching. After 72 hours, the extractions were examined under light microscope and the new hatched second stage number were counted / ml.

3. Effect of *F. oxysporum* , *R. solani* and *Meloidogyne* spp. alone or in combination on potato:

The effect of *F. oxysporum*, *R. solani* and mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) alone or in combination on potato cv. Nicola was carried out under greenhouse conditions. One seed of Nicola potato cultivar was planted in a plastic pot (25 cm in diameter) filled with sterilized clay-sand mixed soil (1 : 1, v/v). After four weeks of planting, the soil was individually infested with *R. solani* and *F. oxysporum* at the rate of 3% of soil weight. Fungi were separately grown on sand wheat-bran medium (1/1), at the same time three thousand of mixed *Meloidogyne* spp. (J₂)

were added by pipetting in 3-4 holes around the root. Treatments were arranged in a completely randomized design with five replicates. Pots were watered as needed and fertilized every two weeks with Greinzet NPK solution (50 ml / 10 L water) added to the soil or sprayed on the leaves.

The effect of *F. oxysporum* alone or associated with mixed *Meloidogyne* spp. and *R. solani* was assessed via disease severity index. A scale of 0-4 was used to assess disease severity where 0 = asymptomatic leaf, 1 = leaf wilted, 2 = leaf with hemiplegic yellowing, 3 = leaf with necrosis, 4 = dead leaf. The incidence of *F. oxysporum* was estimated 45, 60, 75 and 90 days after planting via the index of leaf damage (ILD) calculated per potato plant following the formula of Beye and Lafay (1985).

Where:

$ILD = \sum \text{notes} / \text{max}$

ILD = Index of Leaf Damage

\sum Notes: total notes

Max: 4 times of developed-leaves number

Three months after planting, tops were cut-off and weighted rated for stem canker on a scale of 0-5 as follow: 0 = no disease symptoms, 1 = brown discoloration of stems, 2 = canker covering 25% of the stem circumference, 3 = 25-75% stems covered by canker, 4 = 75% covered by stem canker, 5 = stem completely nipped off or death of plant). Tubers were separated manually from pots and black scurf was assessed on a scale of 0-5 as follows: 0 = no visible sclerotia, 1 = sclerotia covering 1% of the skin-covered tuber surface, 2 = 2-5% covered tuber with sclerotia, 3 = 5-10% covered, 4 = 10-15% covered, 5 = \geq 15% tuber covered by sclerotia (Marin and Robert 2005).

Second stage (J₂) were extracted from soil by sieving modified Baermann technique (Goody, 1957) then counted per pot. Roots were washed under running tap water to remove soil

particles, and then weighed. Females and egg-masses as well as number of developmental stage per root were counted by staining root in sodium hypochlorite-acid fuchsin according to (El-Hazmy, 1992). The rate of nematode reproduction (PF / PI) was calculated according to (Norton, 1978) where $\text{Reproduction Rate} = \text{Final population (PF)} / \text{Initial population (PI)}$.

4. Statistical analysis:

Data were subjected to analysis of variance (ANOVA). The mean differences were evaluated for their significant level by Duncans Multiple Range Test (DMRT). The analysis of data was done by using Costat software.

RESULTS

Interaction between *Fusarium oxysporum*, *Rhizoctonia solani* and *Meloidogyne* spp. on potato plant cv. Nicola:

A. The effect of interaction on *Fusarium* wilt severity:

Data in Table (1) show significant variation of *Fusarium* wilt severity on the potato plants cv. Nicola inoculated with *Fusarium oxysporum* alone or in combination with mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) and *Rhizoctonia solani*. The severity was measured by Index of Leaf Damage (ILD) after 45, 60, 75 and 90 days of planting. The highest wilt severity was recorded by *F. oxysporum*, mixed *Meloidogyne* spp. and *R. solani* followed by *F. oxysporum* and mixed *Meloidogyne* spp., while the least wilt severity was recorded by *F. oxysporum* alone.

B. Effect of interaction on black scurf and stem canker incidence:

Data in Table (2) show that combination of *Rhizoctonia solani* with mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) or *Rhizoctonia solani*, mixed *Meloidogyne* spp. and *Fusarium oxysporum* increased the

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infected tuber, stem canker and black scurf incidence compared to *Rhizoctonia solani* alone. The percentage of infected tubers was 69.06% (*R. solani*, *F. oxysporum* and mixed *Meloidogyne* spp.) and was 62.16% (*R. solani* and mixed *Meloidogyne* spp.), while it was 56.00% in the case of *R. solani* alone. Data also clear that interaction between *R. solani* and other tested organisms increased

stem canker and black scurf incidence compared with *R. solani* alone. Stem canker incidence was 4.21 (*R. solani*, *F. oxysporum* and mixed *Meloidogyne* spp.) and 3.33 (*R. solani* and mixed *Meloidogyne* spp.). Black scurf incidence was 4.02 (*R. solani*, *F. oxysporum* and mixed *Meloidogyne* spp.) and 3.86 (*R. solani* and mixed *Meloidogyne* spp.).

Table (1). Index of leaf damage (ILD) of potato plant cv. Nicola as affected by *Fusarium oxysporum* separately or in combination with *Meloidogyne* spp. and *Rhizoctonia solani* under greenhouse conditions.

Treatment	Days after planting / ILD			
	45 days	60 days	75 days	90 days
<i>Fusarium oxysporum</i>	0.376 ^b	0.564 ^c	1.344 ^c	2.167 ^c
<i>F.oxysporum</i> + mixed <i>Meloidogyne</i> spp.	0.413 ^{ab}	0.741 ^b	1.619 ^b	2.318 ^b
<i>F.oxysporum</i> + <i>R.solani</i> + mixed <i>Meloidogyne</i> spp.	0.474 ^a	0.876 ^a	1.921 ^a	2.810 ^a
Control	0 ^c	0 ^d	0 ^d	0 ^d
LSD 5%	0.07	0.04	0.06	0.08

* Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($p \leq 0.05$).

* Index of Leaf Damage (ILD) = \sum notes / max.
 ILD = Index of Leaf Damage.
 \sum Notes: total notes.
 Max: 4 times of developed – leaves number.

Table (2). Effect of *Rhizoctonia solani* alone or in combination with *Fusarium oxysporum* and *Meloidogyne* spp. on black scurf and stem canker of potato cv. Nicola under greenhouse conditions.

Treatment	Infected tuber (%)	Healthy tuber (%)	Stem canker (0-5)	Black scurf (0-5)
<i>Rhizoctonia solani</i>	56.00 ^b	44.00 ^b	2.9 ^b	3.5 ^b
<i>R.solani</i> + mixed <i>Meloidogyne</i> spp.	62.16 ^{ab}	37.84 ^{bc}	3.33 ^b	3.86 ^a
<i>R.solani</i> + <i>F.oxysporum</i> + mixed <i>Meloidogyne</i> spp.	69.06 ^a	30.94 ^c	4.21 ^a	4.02 ^a
Control	0 ^c	100 ^a	0 ^c	0 ^c
LSD 5 %	11.74	11.74	0.74	0.33

* Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($p \leq 0.05$).

Stem canker scale of 0-5 as follow:

0 = no disease symptoms.

2 = canker covering 25% of the stem circumference.

3 = 25-75 % stems covered by canker.

5 = stem completely nipped off or death of plant).

Black scurf scale of 0-5 as follows:

1 = brown discoloration of stems.

4 = 75 % covered by stem canker.

0 = no visible sclerotia.

1 = sclerotia covering 1% of the skin-covered tuber surface.

2 = 2-5% covered tuber with sclerotia.

4 = 10-15% covered.

3 = 5-10% covered.

5 = \geq 15% tuber covered by sclerotia.

C. Effect of interaction on root-knot nematode reproduction:

Data presented in Table (3) indicate that the tested pathogenic fungi significantly reduced all parameters of mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) i.e., number of J₂ / pot, developmental stages, No. of females, egg masses and reproduction factor. The highest reduction of all parameters was recorded by mixed *Meloidogyne* spp., *R. solani* and *F. oxysporum* interaction. The least reduction was recorded by mixed *Meloidogyne* spp. and *R. solani* interaction. The lowest reproduction factor was 4.83 in mixed *Meloidogyne* spp., *R. solani* and *F. oxysporum* interaction, 5.14 in mixed *Meloidogyne* spp. and *F. oxysporum* interaction, while it was 5.38 in mixed *Meloidogyne* spp. and *R. solani* interaction.

D. Effect of interaction on plant parameters:

Data in Table (4) show that all treatment of pathogenic organisms (separately or in combination) significantly reduced plant growth parameters compared to control. The highest effect on plant growth was recorded by *F. oxysporum*, *R. solani* and mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) interaction, the root weight was 10.50 g, shoot weight 62.78 g, tuber weight 84.94 and plant height 36.62 cm. The least effect was recorded by mixed *Meloidogyne* spp. treatment, the root weight was 15.98 g, shoot weight 74.12 g, tubers weight 110.28 g and plant height 44.12 cm. It can be concluded from the results in Table (4) that, interaction between root-knot nematode and pathogenic fungi was more effective in reducing plant parameters compared to any of the pathogenic organisms alone.

DISCUSSION

In the present study, it was observed that *Fusarium* wilt severity was increased by the presence of nematode alone or nematode and *R. solani* together. These results may be due to alteration of the host physiology and biochemistry (Khan and Hosseini, 1991). On the same trend, Roberts *et al.* (2006) reported that, wilt incidence on cotton was increased by interaction with nematode and *Fusarium* wilt agent. The present study show also that combination of *R. solani* with mixed group of *Meloidogyne* spp. (*M. javanica* and *M. incognita*) or with mixed *Meloidogyne* spp. and *F. oxysporum* increased infected tubers with stem canker and black scurf incidence. These results are in agreement with Karlsoon (2006), who indicated that synergistic interactions between plant parasitic nematodes and *R. solani* cause more quantitative and qualitative yield losses than either pathogen acting alone. It was noticed that the combined infection with the nematode and fungi decreased all nematode parameters. This result may be due to production of fungal toxins, adverse effect of the fungi on the nematode penetration and / or fungal invasion of giant cells which disrupts nematode feeding (Makbel, Asmaa *et al.*, 2007). The results also revealed that all pathogenic organisms caused significantly reduction on various plant growth parameters. The synergistic effects of mixed *Meloidogyne* spp. and *F. oxysporum* or *R. solani* on potato plants were more greater than the effect of either pathogen alone. Previous study by Senthamarai *et al.* (2008) indicated that nematodes cause injury on root surface, weakening the root tissue, thereby making host plant more prone to fungal attack so a greater damages were observed on

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plant growth.

Table (3). *Meloidogyne* spp. reproduction on potato plant cv. Nicola by infection with nematode separately or in combination with *Fusarium oxysporum* and *Rhizoctonia solani* under greenhouse conditions.

Treatment	No of (J ₂) soil / pot	No of developmental stage (J ₂ , J ₃ , J ₄)	No of female	No of egg masses	Reproduction factor (Pf / Pi)*
Mixed <i>Meloidogyne</i> spp.	20448 ^a	460 ^a	148 ^a	167 ^a	7.07 ^a
Mixed <i>Meloidogyne</i> spp. + <i>F.oxysporum</i>	14880 ^c	339 ^c	118 ^b	127 ^c	5.14 ^c
Mixed <i>Meloidogyne</i> spp. + <i>R.solani</i>	15552 ^b	357 ^b	124 ^b	136 ^b	5.38 ^b
Mixed <i>Meloidogyne</i> spp. + <i>R.solani</i> + <i>F.oxysporum</i>	14016 ^d	304 ^d	100 ^c	108 ^d	4.83 ^d
Control	0 ^e	0 ^e	0 ^d	0 ^e	0 ^e
LSD 5%	473.88	10.12	6.52	5.93	0.15

* Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($p \leq 0.05$).

• (Pf): Final population
(Pi): Initial population

Table (4). Influence of *Fusarium oxysporum*, *Rhizoctonia solani*, *Meloidogyne* spp. separately or in combination on some parameters of potato cv. Nicola under greenhouse conditions.

Treatment	Root weight (g)	Shoot weight (g)	Tuber weight (g)	Plant height (cm)
<i>Fusarium oxysporum</i>	14.42 ^c	70.7 ^d	101.84 ^d	41.46 ^{cd}
<i>Rhizoctonia solani</i>	14.84 ^{bc}	72.16 ^c	106.32 ^c	42.7 ^c
Mixed <i>Meloidogyne</i> spp.	15.98 ^b	74.12 ^b	110.28 ^b	44.12 ^b
<i>F.oxysporum</i> + mixed <i>Meloidogyne</i> spp.	12.84 ^d	66.64 ^f	93.08 ^f	38.40 ^e
<i>R. solani</i> + mixed <i>Meloidogyne</i> spp.	13.04 ^d	69.20 ^e	96.08 ^e	40.42 ^d
<i>F.oxysporum</i> + <i>R.solani</i> + mixed <i>Meloidogyne</i> spp.	10.50 ^e	62.78 ^g	84.94 ^g	36.62 ^f
Control	22.30 ^a	89.5 ^a	147.12 ^a	55.22 ^a

LSD 5%	1.23	1.40	1.58	1.35
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* Duncan's multiple range test was used. Values followed by the same letters are not significantly differed ($p \leq 0.05$).

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ونيماتودا تعقد الجذور *Meloidogyne* spp. على نباتات البطاطس

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الملخص العربى :

تم دراسة تأثير كل من الفطرين *R. solani* و *F. oxysporum* ونيماتودا تعقد الجذور *Meloidogyne* spp. منفردين أو مجتمعين على نبات البطاطس صنف نيقولا تحت ظروف الصوبة . وقد أوضحت النتائج حدوث زيادة معنوية لمعدل حدوث الذبول لنباتات البطاطس فى حالة إضافة النيماتودا أو النيماتودا وفطر *R. solani* مع الفطر المسبب للذبول (*F. oxysporum*) مقارنةً بالمعاملة بفطر الذبول فقط . كما أوضحت النتائج أن نسبة الإصابة ومعدل الإصابة للدرنات بمرض القشرة السوداء ومرض تقرح الساق قد زاد بصورة معنوية عند المعاملة بالنيماتودا أو النيماتودا وفطر *R. solani* مع فطر *F. oxysporum* مقارنةً بالمعاملة بفطر *R. solani* فقط . وقد لوحظ حدوث نقص معنوى فى كل صفات النيماتودا تحت الدراسة (عدد الطور اليرقى الثانى فى التربة – عدد كتل البيض – عدد الإناث – عدد الأطوار المختلفة للنيماتودا – معدل التكاثر) عند إضافة كل من الفطرين *R. solani* و *F. oxysporum* للنباتات المعاملة بالنيماتودا

مقارنةً بمعاملة النيमतودا فقط . كان لمعاملة نبات البطاطس بالمسببات المرضية مجتمعة تأثير كبير في حدوث نقص معنوى لكلٍ من وزن الجذور ، وزن المجموع الخضرى ، وزن الدرناات ، وارتفاع النبات مقارنةً بهذه القياسات فى النباتاات بالمعاملة بكلٍ مسبب مرضى على حده .