Relationship between Root-Rrot / Wilt Fungi and Root-Knot Nematode on Disease Incidence, Growth of Plants and Reproduction of Nematode on Alfalfa.

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Abstract: *Interrelationship between **Rhizoctonia solani**, **Fusarium oxysporum f.sp. medicaginis**, **Verticillium alboatrum** and **Meloidogyne incognita** were studied on two alfalfa cultivars i.e. Sewa and Cw 8959. The results obtained indicated that **R. solani**, **F. oxysporum f.sp. medicaginis or V. albo-atrum** affected more when combined with root-knot nematode (**M. incognita**) than when they were applicated individually.*

*The percentage of post emergence damping-off was increased by the combination of fungi and root-knot nematode as compared with the fungi alone. **R. solani** was effective when combined with **M .incognita** followed by **F. oxysporum** while **V. albo-atrum** showed the least effect when combined with **M. incognita**. In contrast, root gall index as number of juveniles of **M. incognita** were decreased by the combination of nematode and fungus compared with nematode alone on the two cultivars under this investigation. The suppression of plant growth parameters was higher in all combination of the two pathogens (nematode and fungi) than by each pathogen alone especially during seedling stage of the two cultivars under investigation.*

INTRODUCTION

Many crops attacked by plant parasitic nematodes and various plant pathogenic fungi. The disease complex between nematode and fungi had been intensevily studied (Pitcher , 1965 & 1978; Melendez and Powell ., 1967; Porter and Powell , 1967; Powell 1971; Bergeson 1972; Khan and Hosseininejad, 1991; Hassanein *et al.* 2001 and Hosseininejad *et al.* 2003.

Meloidogyne spp. are most common nematode associated with nematode fungi interaction. Several root- knot nematode species , including ***M. incognita*** , ***M. javanica*** and ***M. hapla*** reportedly interact with ***Fusarium*** **spp.** in plant disease complex (Bergeson *et al.*, 1970; Garber *et al.* , 1979; Hulton *et al.* , 1973; Jonhson *et al.* , 1969; Morrell *et al.* , 1981; Thomason *et al.* , 1957 and Yang *et al.* , 1976 and Hosseininejad, 1995 and 1999) .

Nematode fungi relationship including ***Meloidogyne*** and ***Fusarium oxysporum*** have been reported in alfalfa. Griffin & Thyr., (1988) concluded that root discoloration was increased and plant growth of alfalfa was significantly suppressed by sequential inoculation with both organisms (***M. hapla*** and ***F. oxysporum***) compared to inoculation with *F. oxysporum* only.

Numerous investigators have detailed relationship between ***Rhizoctonia solani*** and nematodes in which their association results in augmented disease incidence and altered nematode reproduction.

Increased incidence of seedling disease caused by ***R. solani*** was noticed in the presence of ***Meloidogyne*** **spp.** on various crops (Carter, 1975; Powell & Batten, 1967 and Reddy *et al.*, 1979).

Also, it has been observed during the survey conducted in Ismailia governorate that the decline of alfalfa stand were obvious in fields infested with ***M. javanica*** and ***F. oxysporum*** or ***R. solani.*** Verticillium wilt disease which is quite similar to Fusarium wilt was influenced by nematodes in some instance (Mountain & Mckeen, 1960 & 1961; Meclellan *et al.*, 1995 and Hosseininejad *et al.* 2003.). They indicated that root-knot nematodes increased damage by Verticillium wilt fungus in some cases.

The objective of this work was to study the effect of infection by 3 fungi, namely ***F. oxysporum***, ***V. albo-atrum*** and ***R. solani*** singly or in combination with the root-knot nematode (***M. incognita***) on disease incidence, plants growth and nematode reproduction on two alfalfa cultivars.

MATERIALS AND METHODS

I – The nematode inoculum preparation:

Population of ***M. incognita*** was obtained from diseased alfalfa plants from Ismailia governorate. Identification of ***M. incognita*** was achieved through the examination of several perineal pattern preparations according to Taylor *et al.* (1995).

Single egg mass of previously identified as ***M. incognita*** female was transferred to 25 cm clay pot filed with steam sterilized sandy loam soil in which tomato seedling cv. pritchard was grown . Inoculated pots were placed in the greenhouse at 25 C° to be used as stock culture for further studies. Infected roots were chopped and used as a source of inoculum.

II – The fungus inoculum preparation:

During the survey and identification of the major organisms causing root-rot and wilt diseases in alfalfa

in Ismailia Governorate, the dominant isolated fungi ***R. solani***, ***F. oxysporum*** and ***V. albo-atrum***) were used in this experiment under greenhouse conditions. Eight inoculation treatments were used: ***M. incognita*** alone (Mi), ***R. solani*** alone (Rs), ***F. oxysporum*** alone (Fo), ***V. albo-atrum*** alone (Va), simultaneous inoculations of Mi and Rs (Mi + Rs), Mi and Fo (Mi + Fo), Mi and Va (Mi + Va) and uninoculated control (C). The fungal inocula were prepared by growing equal disk (5 mm diameter), 7 days old cultures of these fungi separately in conical flasks (250 ml)each containing 100 ml sterilized potato-dextrose broth medium at 25 ± 2 C° for two weeks. After incubation the mycelial mat was blended in 100 ml water. Soil infestation was carried out by adding 50 ml fungal suspension/ pot (25 cm in diameter), each containing 5 Kg formalin sterilized soil. Each pot received 3000 newly hatched juveniles (T2) of ***M. incognita*** through four holes around the plants.

All pots were arranged in a completely randomized design and kept under greenhouse conditions at about 25 – 28 C°. Surface sterilized seeds of cv, Sewa and Cw 8959 of alfalfa were sown (20 seeds per pot) on the middle of November. Four replicates were used for each treatment. Pre-, post-emergence damping-off and survival plants were recorded and the pots were depotted and plants were washed free of soil for assessing fungal infection and nematode count. Roots were gently separated from soil washed with a gentle flow of water and dried by pressing lightly between blotting paper. Number of root galls and egg masses were determined. Root galls and egg masses were rated on a scale of 0 to 5 according to Taylor and Sasser *et al.* (1978) and Sasser *et al.* (1984).

Number of second stage juveniles was determined in 250 g. soil taken from each pot. Percentage of infection and discoloration, shoot and root weight were recorded. All data were subjected to statistical analysis and means were compared using least significant difference (L.S.D) test at P = 0.05.

RESULTS AND DISCUSSION

Data in Table (1) revealed that ***R. solani*** had the highest effect in reducing survival plants of the two cultivars of alfalfa either alone or in combination with ***M. incognita***, ***F. oxysporum*** also affected but less than ***R. solani***, while ***V. albo-atrum*** showed lowest effect.

In general, post-emergence damping-off percentages were increased with the infection of the three fungi ***R. solani***, ***F. oxysporum*** and ***V. albo-atrum*** respectively combined with ***M. incognita*** than the single infection of each fungus alone. Post-emergence damping off percentages of the three fungi recorded 6.25, 35.00 and 21.25 % by the single application respectively and increased to 12 .50, 43.75 and 33.75 % when combined each fungus with ***M. incognita*** on Sewa cultivar .The same trend were observed with Cw 8959 cultivar.

The number of survival plants decreased by combined infection with each fungus and ***M. incognita.*** While the percentages of survival plants reached 46 .25, 57.50 and 73.75% for ***R. solani***, ***F. oxysporum*** and ***V. albo-atrum*** respectively by the single application, and decreased to 40.00, 48.75 and 61.25 % by the combined treatments between each fungus and ***M. incognita*** on Sewa cultivar. The same trend was observed with Cw 8959 cultivar. These data are in agreement with those obtained by Ibrahim *et al.* (1982) on cotton and Jonathan and Rajendra (1988) on banana who indicate that nematode predisposes the plant to infect with fungi. Also, El-Sherif *et al.* (1988) reported that the nematode ***M. incognita*** enhanced the infection with ***F. oxysporum*** by 40 %, ***R. solani*** 60 % and ***V. albo-atrum*** by 36 % respectively. Hassanein *et al.* (2001) reported that when the plants infected with nematode they become more liable to infect with *Fusarium*.

Data in Table (2) revealed that the combination between ***M. incognita*** and each of three fungal species generally decreased the root galling index as well as the nematode reproduction on the two alfalfa cultivars. While nematode treatment can caused root gall index (3.5) by the single application. This value decreased to 2.0, 2.3 and 2.8 by the combined application of nematode and each of ***R. solani***, ***F. oxysporum*** and ***V. albo-atrum***, respectively.

Number of ***M. incognita*** juveniles in soil had been also affected by the combined application. The number of juveniles reached 656.0 / 250 g. soil by the single treatment of nematode alone and decreased to 275.0, 450.0 and 575.0 / 250 g. soil when the treatment combined nematode and each of the three fungal species respectively on the cultivar Sewa. The same trend was observed by the cultivar Cw 8959. These data can be explained by the interpretation of Jonathan and Rajendran, (1988) who cited that probably the fungal infection affect the tissue physiologically and rendering them less suitable for nematode colonization and reproduction. These results had been canfirmed by the findings of other investigators. In a study on the interaction between ***Rhizoctonia solani*** and ***Meloidogyne javanica***, Metho *et al.*, (1990) reported that ***R. solani*** reduced the number of galls and egg-masses per plant, as well as the number of eggs -mass of ***M. javanica*** on *Phaseolus vulgaris*.

Fazai *et al.*, (1994) reported that the presence of ***F. oxysporum*** f,sp ***lentis*** on lentil affected the rate of ***Meloidogyne incognita*** multiplication and galling were significantly reduced. Hassanein *et al.*, (2001) cited that the nematode reproduction was adversely affected when preceded by *Fusarium* or when both are inoculated simultaneously combared with those found when nematode exist alone or precede *Fusarium*.

Data in Table (3) reveal that the most effective fungus in reducing the plant growth of alfalfa was ***R. solani*** which reduce weight of root system by 54.6 & 43.3 % for the two cultivars under investigation and this reduction increased to 59.8 & 48.5% by the combination with ***M. incognita***. Also ***Fusarium oxysporum*** caused a reduction in root weight by 30. 9 & 26.8 % for the two cultivars under investigation and this reduction increased to 31.9 & 32.5 % by the combination between ***F. oxysporum*** and ***M. incognita***. The same trend was recorded by ***V. albo-atrum*** which caused the least effect on the two cultivars. The same trend was recorded on the shoot weight of alfalfa by each of the three fungi. The combination between each of the three fungi with root-knot nematode increased their effectiveness to reduce the root and shoot system compared with the infection of nematode alone which caused the least reduction in plant growth. These results are in accordance with the findings of many other investigators on other crops. Ibrahim *et al.* (1982) reported that the presence of ***M .incognita*** enhanced the incidence and severity of wilt on cotton ***F.oxysporum*** **f.sp.*vasinfectum***. Griffin and Thyr (1988) concluded that root discoloration caused by ***F. oxysporum*** **f.sp. *medicaginis*** was increased on alfalfa plants and plant growth was significantly suppressed by sequential inoculation of ***M. hapla*** and ***F. oxysporum*** **f.sp. *medicaginis***. Hassanein *et al.* (2001) reported that synergistic interaction occurred between ***M. incognita*** and ***F. oxysporum*** **f.sp. *fabae*** in concomitant and sequential inoculations , results in significant reduction in plant growth . Also, they indicated that the root and shoot weight are similarly affected following the same trend. Root weight reduction ranged from 8.3 to 88.1% and the range for shoot weight reduction extended from 6.1 to 91.8%.

Increased incidence of seedlings disease caused by ***R. solani*** was noticed in the presence of ***Meloidogyne*** **spp.** on various crops (Powell & Batten, 1967; Cartet, 1975 and Reddy *et a.l*, 1979). Also, Jonathan *et al.* (1988) reported that ***M. incognita*** and ***F. oxysporum*** **f.sp. *cubense*** individually caused significant reduction in shoot weight and root weight of banana plants.

The above mentioned results confirm the importance of nematode control to avoid the aggravation of infection with soilborne fungi. Further attention should be paid to understand the relationship between nematode and other pathogens.

Table (1) Effect of some pathogenic fungi alone or in combination with *M. incognita* on Pre-, post-emergence damping-off and Survival plants of Sewa and Cw 8959 alfalfa cv.

Treatments	Sewa			Cw 8959			% of infection and discoloration	
	Pre-	Post-	Survival	Pre-	Post-	Survival	Sewa	Cw 8959
Rhizoctonia solani **(Rs)**	47.50	6.25	46.25	52.50	11.25	37.50	46.13	49.60
Fusarium oxysporum **(Fo)**	7.50	35.00	57.50	10.00	33.75	57.50	31.65	25.33
Verticillicum albo-atrum **(Va)**	5.00	21.25	73.75	5.00	32.50	62.50	26.30	33.25
Meloidogyne incognita **(Mi)**	2.50	11.25	87.50	7.50	5.00	87.50	23.35	21.25
Rs + Mi	47.50	12.50	40.00	52.50	13.75	33.75	62.60	59.73
Fo + Mi	7.50	43.75	48.75	10.00	38.75	51.25	47.50	48.75
Va + Mi	5.00	33.75	61.25	5.00	42.50	52.50	38.80	46.25
Control	2.50	0.00	97.50	1.25	1.25	97.50	0.00	0.00
L.S.D at 5%: C =	N.S	0.54	N.S	0.17				
T =	3.34	2.58	4.54	0.49				
C x T =	N.S	3.65	6.42	0.69				
T = Treatment								
C = Cultivars								

Table (2): The single and combined effect of *Meloidogyne incognita*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Verticillium albo-atrum* on nematode reproduction in two alfalfa cultivars.

Treatments	Alfalfa cultivars			
	Sewa		Cw 8959	
	R.G.I	J / 250 g soil	R.G.I	J / 250 g soil
Rhizoctonia solani	0.00	0.00	0.00	0.00
R. solani + M. incognita	2.00	275.00	1.8	219.00
Fusarium oxysporum	0.00	0.00	0.00	0.00
F. oxysporum + M. incognita	2.30	450.00	2.5	238.00
Verticillium albo-atrum	0.00	0.00	0.00	0.00
V. albo-atrum + M. incognita	2.8	575.00	2.3	269.00
Meloidogyne incognita	3.5	656.00	3.00	335.00
Control	0.00	0.00	0.00	0.00
L.S.D at 5 %	0.43	4.74	0.48	4.12

(R.G.I = Root gall index. J / 250 g soil = Number of juveniles / 250 g soil).

Table (3): The single and combined effect of *M. incognita*, *R. solani*, *F. oxysporum* and *V. albo-atrum* on the growth of two alfalfa cultivars.

Treatments	Reduction %							
	Sewa				Cw 8959			
	Shoot-weight (g)	R %	Root-weight (g)	R %	Shoot-weight (g)	R %	Root-weight (g)	R %
R. solani	6.20	31.50	2.79	54.6	7.48	22.5	3.48	43.3
R. solani + M. incognita	5.50	39.20	2.47	59.8	5.73	40.6	3.25	48.5
F. oxysporum	7.14	21.00	4.42	30.9	7.84	18.8	4.62	26.8
F. oxysporum + M. incognita	6.92	23.5	4.18	31.9	6.70	25.1	4.26	32.5
V. albo-atrum	7.57	16.4	4.71	21.6	8.22	14.8	5.00	20.8
V. albo-atrum + M. incognita	7.10	21.5	4.49	26.8	7.86	16.5	3.99	36.8
M. incognita	7.89	12.8	5.19	15.5	8.94	7.4	5.73	9.2

(R %) = Reduction percentages compared to the control.

L.S.D at 5 %:	C =	0.17	0.09	0.43
	T =	0.49	0.57	0.61
	C x T =	0.69	0.80	0.86

T = Treatments

C = Cultivars

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أثر العلاقة بين فطريات عفن الجذور والذبول في البرسيم الحجازي ونيماتودا تعقد الجذور

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أجرى هذا البحث فى محطة البحوث الزراعية بالإسماعيلية لدراسة تأثير إضافة نيماتودا تعقد الجذور إلى تربة محقونة بفطريات الريزوكتونيا سولانى و الفيوزاريوم أوكسيسبورم و الفيرتسيليوم البواترم على نسبة الاصابة فى صنفين من أصناف البرسيم الحجازى.

وقد أوضحت النتائج أن إضافة النيماتودا الى التربة المحقونة بكل من الفطريات السابق ذكرها أدى الى زيادة نسبة موت البادرات مقارنة بالعدوى بكل من النيماتودا والفطر على حدة وقد سجل فطر الريزوكتونيا أعلى نسبة إصابة يليه فطر الفيوزاريوم ثم الفيرتسيليوم.

أثبتت النتائج أيضا أن وجود النيماتودا مقترنة بالفطريات السابقة أدى الى انخفاض عدد يرقات النيماتودا. كما لوحظ أيضا أن لوجود النيماتودا مع الفطريات تأثير أكبر على خفض نسبة المحصول مقارنة بوجود كل منهم على حدة.