J. Agric. Sci. Mansoura Univ., 27 (7): 4671 - 4682, 2002

ROLE OF *Trichoderma* SPP. IN CONTROLLING OF *Rotylenchulus reniformis* NEMATODE AND *Fusarium oxysporum* FUNGUS DISEASE COMPLEX INFECTING SUNFLOWER.

Haggag, Wafaa M. ** and A. W. Amin *

* **Dept. of Agric. Zoology and Nematology , Fac. of Agric. , Cairo Univ.**

** **Dept. of Plant Pathology, National Research Center, Dokki, Cairo, Egypt**

ABSTRACT

The effect of spore suspensions and culture filtrates of five *Trichoderma* spp. on controlling reniform nematode, *Rotylenchulus reniformis* and wilt fungus, *Fusarium oxysporum*, disease complex, and also on growth of sunflower plants was studied *in vitro* and under greenhouse conditions. Two weeks old sunflower seedlings cv. Giza 1 treated with each of spore suspension or culture filtrate of *Trichoderma harzianum*, *T. viride*, *T. koningii*, *T. reesei* or *T. hamatum* had highly significant effect on controlling nematodes infection and disease incidence on sunflower roots. *T. hamatum*, *T. harzianum* and *T. koningii* culture filtrates gave a highly significant reduction ($P<0.01$) *in vitro* and decreased the population of female nematodes on sunflower roots. The same results were observed when pots were treated with spore suspension of *Trichoderma* species alone, or in combination with *F. oxysporum*.

Sunflower plants grown in infested soil with *F. oxysporum* and *R. reniformis* mix inoculum showed severe wilt disease than in soil infested with *F. oxysporum* alone . Treatment of *Trichoderma spp.* led to inhibit *Fusarium* growth *in vitro* and also in infested soil with *R. reniformis* and improved significantly the plant growth parameters. Use of *T. hamatum*, *T. harzianum* and *T. koningii* gave the greatest reduction of disease incidence caused by *Fusarium* and/or *R. reniformis* infestation. Generally, there were highly significant reduction ($P<0.01$) in the number of nematode population and Fusarium wilt disease on sunflower and increasing in plant growth parametes when treated with *Trichoderma* species. It can be summarized that the role of *Trichoderma* spp. in controlling of *R. reniformis* and *F. oxysporum* as direct effect by toxic metableolites, direct parasitism on the pathogens and improve plant growth.

Keywords: Fungi, *Fusarium oxysporum*, Nematodes, Sunflower, *Rotylenchulus reniformis, Trichoderma spp.*

INTRODUCTION

Sunflower plants, *Halianthus annus* L. is one of the most important oil crops in Egypt, as well as allover the world. The reniform nematode is the major and serious pests of sunflower that significantly reduce the yield quantity and quality (Amin and Youssef, 1997 and 1998).

Wilt diseases caused by *F. oxysporum* are the most important disease-affecting sunflowers plants. *Fusarium*-nematode interactions are known to decrease the quantity and quality of major world crops including sunflowers. Because of hazards involved in the use of pesticides, biological control of plant parasitic nematodes and soil borne diseases had received increasing attention as a promising supplement in controlling these diseases

(Amin, 1999, Ara *et al.*, 1996 and Stephan *et al.*, 1996). Agrochemical pesticides have successfully been used for a long time but due to their environmental pollution, its necessary for planning new methods of control strategies. Biological control using certain biocontrol agents gave satisfactory results (Chen *et al.*, 2000 and Nicola and George, 2000).

Of the various bio-agents, fungi of *Trichoderma* species have been known to suppress many soil borne pathogens and nematodes under greenhouse and field conditions by toxic metabolites production (Ghaffar, 1995 and Parveen *et al.*, 1993). *Trichoderma harzianum*, *T. hamatum* has been found to antagonize fungal plant pathogen and parasitic nematodes (Siddiqui *et al.*, 1999). Spiegel and Chet (1998) found that *T. harzianum* and *T viride* are consistently effective, practical and economical and can serve as a model as a biocontrol agents against soil borne pathogens and plant parasitic nematodes in integrated pest management programs.

The present study aimed to study the effects of spore suspension and culture filtrates of *Trichoderma* species on controlling *F. oxysporum – R. reniformis* disease complex, and on plant growth of sunflower plants cv. Giza 1 under greenhouse conditions.

MATERIALS AND METHODS

The fungus, *Fusarium oxysporum* was isolated from diseased sunflower plants, identified in Plant Pathology Department, National Research Center, Egypt and cultured on Potato Dextrose Agar (PDA).

Spore suspension of the fungus was harvested and adjusted to $1X10^5$ spores /ml. Biocontrol agents, *Trichoderma* spp. were isolated from soil rhizosphere of sunflower plants and maintained on PDA. Culture filtrates of *Trichoderma* spp. were prepared by growing in 250 ml flasks containing 50 ml of malt extract agar (MA) for 15 days at 25 C° ±1. Culture filtrates were collected under sterilized condition. Five, 10 and 15 ml of spores free cultural filtrates were added to Petri-dishes containing PDA medium to give final concentrations of 25, 50 and 75 % respectively. Petri dishes were inoculated with equal disks - 5mm in diameter -of *Fusarium*. Colony diameters of *Fusarium* were measured and calculated as percentage of control treatment. Seeds of sunflower (*Helianthus annus* L.) cv. Giza 1 were sown in 15 cm diameter pots filled with one kg sandy loam soil (1:1 v/v) free of plant pathogen and parasitic nematodes. After germination (about two weeks), the plants in each pot were thinned to one plant/pot. Pots were divided into two sets and two experiments were carried out.

A 17.5 ml of each fungal filtrate were mixed with 2.5 ml of nematode suspension (gave final concentration of 50% fungal filtrate) containing 2000 un-swollen females of *R. reniformis* (Linford and oliviera) in 90 mm Petri-dishes. As a check treatment 17.5 ml distilled water was added with 2.5-ml nematode suspension. Four replicates were used for each treatment. Active and non-active nematode counts were recorded *in vitro* after two and 7 days and checked *In vivo* on sunflower roots after one-week exposure. Nematodes in Petri-dishes have been washed through using current tap water. Then,

2000 un-swollen females were pipetted in 5 holes around two weeks old sunflower roots in 15-cm diameter plastic pots. The plants were left to grow for 10 weeks.

Two days before nematode inoculation, soils were infestation with $3X10^3$ colony forming units (cfu) g^{-1} of *F. oxysporum* as assessed soil dilution technique. Ten ml of spore suspension were pipetted around seedling roots in pots. Then, 10 ml of either conidial suspensions ($3X10^5$ spore/ml) or culture filtrates (100%) of *T. harzianum* (Raifi), *T viride* (Pers.), *T. koningii* (qudem), *T. reesei* (Simmons) and *T. hamatum* (Bon.) were added around seedling roots. Each pot was inoculated with 2000 infective stages of *R. reniformis* (juveniles and un-swollen females) in five holes around two weeks old sunflower roots in 15-cm diameter plastic pots. Un-inoculated - untreated four pots were served as check plants and other four pots inoculated - untreated served as check nematode. Four replicates were used for treatment. Pots were arranged in a complete randomized design in a greenhouse at 30±5C° and watered daily.

Population counts of *Fusarium* (Nash and Snyder) and *Trichoderma* species were assessed by soil dilution technique and expressed as cfu. During nine weeks of growth, percentage of reniform nematode and disease complex incidence was recorded.

Wilt disease was recorded as percentage of total disease and on scale (0-5) as disease syndrome.

After ten weeks of nematodes infestation, sunflower plants were carefully uprooted and nematodes in soil and roots were counted (Franklin, 1949). The number of nematodes in soil, females and egg-masses in roots of *R. reniformis* compared to untreated pots was calculated. Length and weight of shoots and roots and flowering disc weight were recorded.

Data were statistically analyzed using New Least Significant Difference (New LSD) and (LSD).

RESULTS

All the tested culture filtrates of *Trichoderma* showed a highly significant effect ($P<0.01$) on larval activity, were the non-active larvae were 93, 89, and 77% for *T. hamatum*, *T. harzianum* and *T. koningii* respectively after 2 days exposure (Table 1). The percentage of non-active larvae increased after 7 days, where, the non-active larvae were recorded 100% for *T. hamatum* and *T. harzianum* followed by *T. koningii* (94%), *T. viride* (86%) and *T. reesei* (77%). When the population nematodes of Petri-dishes tested *in vivo* on sunflower roots, the highest reduction in the number of females reached to 100% for *T. hamatum* and *T. harzianum* and 92.4% for *T. koningii* (Table 2).

Using of *Trichoderma* spore suspension alone or in combination with *F. oxysporum* gave a highly significant ($P<0.01$) reduction of females and egg-masses of *R. reniformis* (Table. 3). Great reduction in reniform nematodes were recorded, where the percentag results reached 84.5%, 67.2% and 58.6% for *T. hamatum T. harzianum* and *T. koningii* , respectively.

Table (1): Effect of *Trichoderma* species culture filtrates (50% concentration) on *Rotylenchulus reniformis* during one-week exposure in Petri dishes.

Treatment	*Rotylenchulus reniformis*			
	No o f active larvae after 2 days	% Non-active larvae after 2 days	No o f active larvae after 7 days	% Non-active larvae after 7days
Check Nematode	100	0	100	0.0
Trichoderma harzianum	11	89	0.0	100
Trichoderma viride	31	69	14	86
Trichoderma koningii	23	77	6	94
Trichoderma reesei	43	57	23	77
Trichoderma hamatum	7	93	0.0	100
LSD 0.05	3.2	3.2	1.9	1.9
LSD 0.01	4.3	4.3	2.6	2.6

Table (2): Effect of *Trichoderma* species culture filtrates in control of *Rotylenchulus reniformis* (R.r) after one-week exposure and their development on sunflower.

Treatment	*Rotylenchulus reniformis*			
	Number of Female	% Females Reduction	Number of Egg-mass	% Egg-masses Reduction
Check Nematode (*Rotylenchulus reniformis*)	105	0.0	96	0.0
***Trichoderma harzianum* + (R.r)**	0	100.0	0	100.0
***Trichoderma viride* + (R.r)**	20	80.9	15	84.4
***Trichoderma koningii* + (R.r)**	8	92.4	2	97.9
***Trichoderma reesei* + (R.r)**	36	65.7	36	62.5
***Trichoderma hamatum* + (R.r)**	0	100.0	0	100
LSD 0.05	20.6	-	6.9	-
LSD 0.01	29.6	-	9.5	-

When *Trichoderma* treated in combination with *F. oxysporum*,the nematode reduction reached to *T. hamatum* (89.7%) and *T. harzianum* (87.9%) followed by *T. viride* (77.6%) and *T. koningii* (56.9%). On the other hand, statistical analysis of the data (Table 3) showed that all the culture filtrates of *Trichoderma* decreased significantly ($P<0.01$) .

The numbers of females, numbers of egg-masses and the total numbers of nematodes in sunflower roots, as compared with control. *T. hamatum* (74.1%) and *T. harzianum* (67.2%), *T. reesei* (60.3%) and *T. koningii* (53.4%) showed the greatest reduction in the total number of females in roots. Application of *Trichoderma* spp. culture filtrates in combination with *F. oxysporum* had the lowest percent reduction in the final population in roots.However, *T. reesei* (72.4%), *T. koningii* (58.6%) and *T. hamatum* (53.4%) gave the greatest results of nematode reduction.

Amended *Fusarium* medium with culture filtrates of *Trichoderma* species resulted as decrease growth rate compared with un-amended medium (Table 4). Culture filtrate of *T. hamatum* at 75% completely inhibited (100%)

Fusarium growth rate. *T. harzianum* and *T. koningii* culture filtrates exhibited various degrees of inhibition of *Fusarium* growth by 97.6% and 92.3% respectively. Sunflower plants grown in infected soil with *F. oxysporum* and/or *R. reniformis* mix inoculum showed severe wilt disease (Table 5).

Table (3): Effect of spore suspensions and culture filtrates of *Trichoderma* spp. on control of *Rotylenchulus reniformis* on sunflower plants.

Treatment	*Rotylenchulus reniformis*				
	Total number of nematodes in soil	Number of Females	Number of Egg-masses	Total number of nematode in root	% of Nematodes Reduction
Check Nematode (R.r)	6713	16	42	58	0.0
Nematode (R.r) + *Fusarium oxysporium*	1752	5	29	34	41.4
Trichoderma harzianum (T.h.) + (R.r)	2423	3	16	19	67.2
Trichoderma viride(T.v.) + (R.r)	2453	2	22	24	58.6
Trichoderma koningii (T.k) + (R.r)	2029	2	22	24	58.6
Trichoderma reesei (T.r) + (R.r)	1577	6	26	32	44.8
Trichoderma hamatum (T.ha) + (R.r)	1739	1	8	9	84.5
(T.h)+ *Fusarium oxysporium* (F.o) + (R.r)	2093	1	6	7	87.9
(T.v) + (F.o) + (R.r)	4376	2	11	13	77.6
(T.k) + (F.o) + (R.r)	1415	5	20	25	56.9
(T.r) + (F.o) + (R.r)	2286	5	23	28	51.7
(T.ha) + (F.o) + (R.r)	1925	1	5	6	89.7
Trichoderma harzianum Filtrate (T.h.F) + (R.r)	5994	4	15	19	67.2
Trichoderma viride (T.v..F) + (R.r)	1829	7	29	36	37.9
Trichoderma koningii (T.k..F) + (R.r)	3855	6	21	27	53.4
Trichoderma reesei (T.r..F) + (R.r)	3964	3	20	23	60.3
Trichoderma hamatum (T.ha..F) + (R.r)	2857	4	11	15	74.1
(T.h.F)+ *Fusarium oxysporium* (F.o) + (R.r)	2450	3	36	39	32.8
(T.v.F) + (F.o) + (R.r)	2449	3	27	30	48.3
(T.k.F) + (F.o) + (R.r)	3124	6	18	24	58.6
(T.r.F) + (F.o) + (R.r)	1672	4	12	16	72.4
(T.ha.F) + (F.o) + (R.r)	1706	3	24	27	53.4
New L.S.D 0.5	349.2	2.3	10.2	11.0	-
New L.S.D 0.1	454.3	3.0	13.2	13.4	-

Table (4): *In Vitro* effect of *Trichoderma* species culture filtrates on *Fusarium oxysporium* growth.

Treatment	Dilution of culture filtrates %		
	25	50	75
Trichoderma harzianum	46.6	82.3	97.6
Trichoderma viride	24.6	45.2	72.3
Trichoderma koningii	34.3	61.5	92.3
Trichoderma reesei	12.6	36.3	66.6
Trichoderma hamatum	56.4	94.3	100
New L.S.D 0.5	9.3	13.3	21.4
New L.S.D 0.1	11.3	15.6	23.9

Table (5): Effect of spore suspensions and culture filtrates of *Trichoderma* spp. in control of Fusarium wilt disease of sunflower plants infested soil with *F. oxysporium* and/or *Rotylenchulus reniformis*.

Treatment	Spore suspensions		Culture filtrates	
	% Disease incidence	Disease scale (0-5)	% Disease incidence	Disease scale (0-5)
Check plant	0.0	0.0	0.0	0.0
Check Nematode (R.r)	0.0	0.0	0.0	0.0
Check Fusarium *oxysporium (F.o)*	27.3	3.3	27.3	3.6
Nematode(R.r)+ *Fusarium oxysporium*	44.3	4.6	44.6	4.6
Trichoderma harzianum (T.h.) + (F.o)	0.0	0.0	0.6	1.3
Trichoderma viride (T.v.) + (F.o)	2.6	1.3	5.3	2.3
Trichoderma koningii (T.k) + (F.o)	0.3	0.6	1.6	1.3
Trichoderma reesei (T.r) + (F.o)	6.6	1.6	9.6	2.3
Trichoderma hamatum (T.ha) + (F.o)	0.0	0.0	0.6	0.6
(T.h)+*Fusarium oxysporium* (F.o) + (R.r)	6.6	1.3	12.3	1.6
(T.v) + (F.o) + (R.r)	16.3	3.0	18.4	2.6
(T.k) + (F.o) + (R.r)	8.6	1.3	9.3	2.3
(T.r) + (F.o) + (R.r)	12.3	3.0	24.2	3.0
(T.ha) + (F.o) + (R.r)	3.6	0.6	6.6	1.3
New L.S.D 0.5	2.2	0.8	3.2	1.3
New L.S.D 0.1	4.3	1.3	5.6	2.6

Treated plants with either spore suspensions or culture filtrates of *Trichoderma* species showed significant reduction in fusarium wilt disease incidence under infested soil with *R. reniformis*. Spore suspension treated plants was more effective on fusarium wilt control than culture filtrate. *T. hamatum* and *T. harzianum* as spore suspensions or culture filtrates showed high level of reduction effect on *Fusarium*-nematode interaction. *Fusarium* counts in rhizosphere soil of sunflower grown in infested soil with *R. reniformis* was higher during plant growth until 9 weeks (Table 6).

Table (6): Effect of spore suspensions and culture filtrates of *Trichoderma* species on *Fusarium* population in soil rhizospere of sunflower plants grown in soil infested with *F. oxysporium* and/or *Rotylenchulus reniformis*.

Treatment	Population counts/ weeks					
	Spore suspensions			Culture filtrates		
	3wk	6wk	9wk	3wk	6wk	9wk
Check plant	0.0	0.0	0.0	0.0	0.0	0.0
Check Fusarium *oxysporium (F.o)*	3.3	4.6	9.8	3.3	4.6	9.8
Nematode(R.r) + *Fusarium oxysporium*	3.9	5.3	14.6	3.9	5.2	14.6
Trichoderma harzianum (T.h.) + (F.o)	0.3	1.3	2.3	0.6	2.3	4.6
Trichoderma viride (T.v.) + (F.o)	2.3	2.6	3.2	3.3	3.6	3.6
Trichoderma koningii (T.k) + (F.o)	1.6	1.6	2.3	2.3	2.3	4.6
Trichoderma reesei (T.r) + (F.o)	2.3	2.8	3.3	3.3	3.6	2.3
Trichoderma hamatum (T.ha) + (F.o)	0.3	0.6	1.3	1.3	1.6	2.3
(T.h)+*Fusarium oxysporium* (F.o)+ (R.r)	1.3	2.3	3.3	2.3	3.3	4.2
(T.v) + (F.o) + (R.r)	2.6	3.3	3.9	3.6	3.9	5.3
(T.k) + (F.o) + (R.r)	2.3	2.3	3.3	2.6	3.6	4.2
(T.r) + (F.o) + (R.r)	2.9	3.6	4.2	4.0	4.3	5.6
(T.ha) + (F.o) + (R.r)	0.6	0.6	1.6	1.6	1.6	2.3
New L.S.D 0.5	0.7	0.9	1.1	0.8	1.1	1.3
New L.S.D 0.1	1.3	1.5	3.6	1.5	1.7	3.6

Seedlings treatment with either spore suspensions or culture filtrates of *Trichoderma* species, resulted in decrease of *Fusarium* counts in soil rhizospere. Spore suspension or culture filtrates of *Trichoderma hamatum* and *T. harzianum* decreased significantly *Fusarium* counts either in *F. oxysporum* and/or *R. reniformis* infested soil. Concerning *Trichoderma* population in nematode fungal infested soil. Data revealed that *Trichoderma* counts were increased in plant rhizosphere during plant growth periods until 9 weeks (Table 7). *T. hamatum, T. harzianum* and *T. koningii* counts were higher in rhizosphere soil than others. The infested soil with *R. reniformis* and/or *F. oxysporum* resulted in stimulated of *Trichoderma* counts. The highest population was obtained in *R. reniformis* infested soil and /or with *F. oxysporum*. Also, *T. hamatum* and *T. harzianum* counts in infested soil with *R. reniformis* and *F. oxysporum* were higher than other treatments.

Table (7): Population counts of *Trichoderma* spp. in soil rhizospere of sunflower grown in soil infested with *F. oxysporium* and/or *Rotylenchulus reniformis*.

	Population counts/week			
	1 wk	3wk	6wk	9wk
Trichoderma harzianum (T.h.)	4.0	8.4	14.6	22.3
Trichoderma viride (T.v.)	2.6	5.6	7.5	9.6
Trichoderma koningii (T.k)	3.6	7.8	9.1	11.6
Trichoderma reesei (T.r)	3.0	4.2	5.2	7.6
Trichoderma hamatum (T.ha)	8.6	10.4	16.4	32.3
(T.h.) + ***Rotylenchulus reniformis*** (R.r)	11.6	18.6	24.3	31.6
Trichoderma viride (T.v.) + (R.r)	8.6	14.6	20.4	20.7
Trichoderma koningii (T.k) + (R.r)	9.6	16.4	18.6	26.7
Trichoderma reesei (T.r) + (R.r)	6.2	7.6	9.4	14.6
Trichoderma hamatum (T.ha) + (R.r)	16.6	20.4	36.6	56.6
(T.h.) + ***Fusarium oxysporium*** (F.o)	6.3	10.6	11.4	16.2
(T.v.) + (F.o)	3.6	6.4	9.3	12.2
(T.k) + (F.o)	7.9	8.4	10.8	15.4
(T.r) + (F.o)	4.3	6.2	8.4	11.6
(T.ha) + (F.o)	10.6	12.6	21.6	36.6
(T.h)+ (F.o) + (R.r)	16.6	23.4	33.6	39.6
(T(T.v) + (F.o) + (R.r)	12.2	16.4	23.6	31.6
(T.k) + (F.o) + (R.r)	15.6	20.2	31.6	36.2
(T.r) + (F.o) + (R.r)	9.6	15.3	14.3	18.6
(T.ha) + (F.o) + (R.r)	20.6	32.6	41.6	64.2
New L.S.D 0.5	3.6	4.6	8.6	6.6
New L.S.D 0.1	5.4	8.2	15.3	13.6

Data indicated that *Trichoderma* species treated plants gave higher in plant length, weight and flower disc weight (Tables 8 and 9). The results revealed that soil infested with either *R. reniformis* and/or *F. oxysporum* significantly decreased the plant length and weight. But, treatment with *Trichoderma* species in combination with *R. reniformis* and/or *F. oxysporum* significantly increased plant length and weight. Also, *T. harzianum* and *T. hamatum* showed the highest effect on different growth parameters. While, a slight increase was observed with *T. viride* and *T. reesei*.

Table (8): Effect of spore suspensions of *Trichoderma* spp. on sunflower growth parameters in infested soil with *Fusarium oxysporium* and/or *Rotylenchulus reniformis*.

Treatment	Plant length (cm)		Plant dry weight (g)		Flower disc weight (g)	
	Root	Shoot	Root	Shoot	Fresh	Dry
Trichoderma harzianum (T.h.)	12.8	86.3	0.6	2.5	1.4	0.5
Trichoderma viride (T.v.)	12.9	83.3	0.5	1.9	1.2	0.4
Trichoderma koningii (T.k)	12.5	80.5	0.6	1.9	1.3	0.4
Trichoderma reesei (T.r)	13.0	86.5	0.5	1.7	1.2	0.4
Trichoderma hamatum (T.ha)	11.8	88.0	0.7	2.4	1.6	0.4
(T.h.)+ *(Fusarium oxysporium* (F.o)	12.8	82.6	0.4	2.6	1.3	0.4
(T.v.) + (F.o)	12.3	73.6	0.4	2.5	1.1	0.4
(T.k) + (F.o)	11.6	71.6	0.4	2.4	1.2	0.4
(T.r) + (F.o)	10.8	70.0	0.5	1.9	1.1	0.4
(T.ha) + (F.o)	12.3	86.0	0.4	2.8	1.6	0.5
(T.h)+ Nematode (R.r)	12.6	85.5	0.4	2.7	1.4	0.4
(T.v) + (R.r)	12.0	76.6	0.4	2.6	1.1	0.4
(T.k) + (R.r)	12.5	80.0	0.4	2.1	1.4	0.4
(T.r) + (R.r)	11.0	74.0	0.5	2.0	1.0	0.3
(T.ha) + (R.r)	12.3	84.3	0.4	2.1	1.7	0.5
(T.h) + (F.o) + (R.r)	11.9	79.8	0.4	2.0	1.2	0.4
(Tv) + (F.o) + (R.r)	11.6	77.3	0.4	1.7	0.8	0.3
(T.k) + (F.o) + (R.r)	11.3	75.5	0.4	2.0	1.1	0.3
(T.r) + (F.o) + (R.r)	10.2	70.1	0.3	1.9	0.8	0.2
(T.ha) + (F.o) + (R.r)	12.0	80.7	0.4	2.2	1.2	0.3
*Check (*Fusrium oxysporium*)*	6.2	59.3	0.2	1.5	0.7	0.2
Check (*Rotylenchulus reniformis* (R.r).	12.3	71.0	0.8	0.5	0.1	0.0
Check (F.o.) + Nematode (R.r)	4.6	45.6	0.9	0.2	0.0	0.5
Check (plant)	8.0	80.0	0.3	2.0	0.7	0.4
New L.S.D 0.5	1.0	4.3	0.2	0.1	0.2	0.1
New L.S.D 0.1	3.3	6.9	0.3	0.3	0.5	0.3

Table (9): Effect of culture filtrates of *Trichoderma* spp. on sunflower growth parameters in infested soil with *Fusarium oxysporium* and/or *Rotylenchulus reniformis*.

Treatment	Plant length (cm)		Plant dry weight (g)		Flower disc weight (g)	
	Root	Shoot	Root	Shoot	Fresh	Dry
Trichoderma harzianum (T.h.)	10.3	88.7	0.64	2.42	1.20	0.48
Trichoderma viride (T.v.)	9.6	79.0	0.45	1.94	0.98	0.4
Trichoderma koningii (T.k)	10.4	83.6	0.60	2.30	1.23	0.46
Trichoderma reesei (T.r)	9.0	74.1	0.40	1.36	0.78	1.36
Trichoderma hamatum (T.ha)	11.6	89.4	0.65	2.80	1.34	1.56
(T.h.)+ *(Fusarium oxysporium* (F.o)	11.0	78.6	0.39	2.49	1.23	0.35
(T.v.) + (F.o)	10.0	71.0	0.34	2.32	0.87	0.28
(T.k) + (F.o)	11.3	76.4	0.38	2.56	1.15	0.33
(T.r) + (F.o)	10.0	70.0	0.35	2.21	0.97	0.25
(T.ha) + (F.o)	11.0	79.4	0.45	2.52	1.30	0.45
(T.h)+ Nematode (R.r)	10.6	72.3	0.35	2.45	1.00	0.30
(T.v) + (R.r)	9.4	68.9	0.30	2.11	0.91	0.28
(T.k) + (R.r)	10.6	73.4	0.35	2.32	1.12	0.30
(T.r) + (R.r)	9.6	65.3	0.30	2.08	1.00	0.28
(T.ha) + (R.r)	10.8	76.0	0.40	2.54	1.25	0.28
(T.h) + (F.o) + (R.r)	9.8	70.3	0.32	1.92	1.24	0.30
(Tv) + (F.o) + (R.r)	7.0	50.1	0.30	1.56	1.81	0.22
(T.k) + (F.o) + (R.r)	8.8	71.3	0.36	1.98	1.41	0.30
(T.r) + (F.o) + (R.r)	9.6	50.1	0.29	1.51	0.88	0.26
(T.ha) + (F.o) + (R.r)	9.4	70.0	0.33	2.22	1.24	0.32
*Check (*Fusrium oxysporium*)*	7.0	56.3	0.24	0.28	0.64	0.23
Check (*Rotylenchulus reniformis* (R.r).	6.2	50.2	0.14	0.20	0.12	0.01
Check (F.o.) + Nematode (R.r)	5.6	50.0	0.12	0.19	0.11	0.01
Check (plant)	8.8	78.6	0.28	0.35	0.72	0.35
New L.S.D 0.5	0.9	5.6	0.20	0.19	0.25	0.13
New L.S.D 0.1	1.9	7.3	0.5	0.4	0.5	0.4

DISCUSSION

In the present study, the addition of *Trichoderma* spp. in controlling disease complex caused by reniform nematodes and fusarium-wilt. *Trichoderma* spp. were effective in decreasing the numbers of females and egg-masses in roots and the total numbers of nematodes in soil, and caused significant reduction in the pathogens and increased plant growth parameters.

Trichoderma culture filtrates and spore suspension treatment showed promising results in controlling the reniform nematode on sunflower plants. These results confirm the report of Stephan *et al.*, (1998). He found that, *T. harzianum* was the most promising biocontrol agents against *Meloidogyne javanica* and improve the yield of tomato and eggplant. Also, Reddy *et al.*, (1996) found that *T. harzianum* in combination with some oil cakes was effective in increasing plant growth and reducing the population of nematodes in soil and roots.

Other report demonstrated a specific effect of *Trichoderma* spp. on the development of the plant parasitic nematodes (Haggag and Amin 2001 and Siddiqui *et al.*, 1999). Saifullah (1996 a and b) observed that 100% of the *Globodera rostochiensis* and *G. pallida* were infected and killed by toxic metableolites released from the *T. harzianum* into the medium after 24 hours exposure. *Trichoderma* spp. are known to produce different toxic metableolites include antibiotics, enzymes and others (Di Pietro, 1995) which protected plants from soil borne and plant pathogens (Wu and Wu, 1998).

In the present study, data showed that *F. oxysporum* mixed with the reniform nematode increased the wilt disease incidence to a great extend than plant infected with the fungus alone. This result can be explained that nematodes predispose the plant pathogen invasion, as reported by Haggag and Amin (2001) and Siddiqui *et al.* (1999). Several species of *Trichoderma* had been reported to suppress soil borne disease fungi included *Fusarium* spp. (Wu and Wu, 1998). *Trichoderma* spp. are known to produce other secondary metabolites such as enzymes. Chitinase enzyme have been considered important in the biocontrol pathogenic fungi at low concentration because of their ability to decay fungal cell walls of which a major component is chitin (Lorito *et al.*, 1993). Because chitin is a component of the eggshell of nematodes, which secreted by the egg, chitinase-producing microorganisms also are effective in destroying nematode eggs.

Recently, Belanger *et al.* (1995) and Cotes *et al.* (1996) has been achieved various chitinases, β-1, glucanase and cellulase from biocontrol fungi, *T. harzianum*, *T. hamatum* and *T. koningii*.These references indicated that *Trichoderma* spp. were highly effective in reducing the population of *R. reniformis*. Gadgil *et al.*, (1995) and Kanotra and Mathur, (1995) found that cellulase , glucanase and glucosidase are main enzymes produce by *T. viride* and *T. reesei*. Rajeshwari *et al.* (1998) found that *T. viride* gave a maximum reduction on nematodes population followed by nematicide, carbofuran. It also hypothesized that the production of nematicidal compounds by *Trichoderma* spp., directly affected the nematode or made rootlets attractive

which might have resulted the reduction in the nematode population. Moreover, other researchers reported that *Trichoderma* spp. act as nematophagous fungi on eggs, larvae and males of cyst nematodes (Susan *et al.*, 1990). The suppression of soil nematodes and borne diseases observed in these investigation may be due to results of an increase in soil *Trichoderma* activity owing to the establishments of healthier rhizosphere environment for the growth of sunflower plants.

Also, in this study, *Trichoderma* spp. significantly increased the plant growth parameters. *Trichoderma* known to produce plant growth hormone or stimulation nutrient uptake which improve plant growth (Haggag, 1998), and improve plant resistance to invasion by the pathogens. It was suggested that the role of *Trichoderma* spp. in controlling nematodes and fungi onto 1). Production of enzymes like chitinase which destroy the pathogenic fungal cell wall or nematodes eggshell. 2). Production of different antibiotics and toxic metabolites which act as direct toxic on the pathogens. 3). Direct parasitism on eggs, immature and mature stages of nematodes, in addition to, produce plant growth hormone which improve plant resistance and growth.

REFERENCES

Amin, A.W. (1999). Nematicidal activity of some aromatic and medicinal plants in controlling *Meloidogyne javanica* and *Helicotylenchus dihystera*. Egypt. J. Agronematol., 3: 125-138

Amin, A.W. and M.M.A. Youssef (1997). Efficiency of certain plant leaves for controlling *Meloidogyne javanica* and *Rotylenchulus reniformis* infecting sunflower in Egypt. Intern. J. Nematol., 7: 198-200.

Amin, A.W. and M.M.A. Youssef (1998). Effect of organic amendments on the parasitism of *Meloidogyne javanica* and *Rotylenchulus reniformis* and growth of sunflower. Pakistan J. Nematol., 16: 63-70.

Ara, J.;S. Ehteshamul- Haque; V. Sultana; R. Qasim and A. Ghaffar (1996). Effect of sargassum seaweed and microbial antagonists in the control of root rot disease of sunflower. Pakistan J. Botany, 28:219-223.

Belanger, R.R.; N. Dufour ; J. Caron and N. Benhamou (1995). Chronological events associated with the antagonistic properties of *Trichoderma harzianum* against *Botrytis cinerea*: Indirect evidence for sequential role of antibiosis and parasitism. Biocontrol Sci. and Technology, 5: 41-53.

Chen, J.; G.S. Abawi and B. M. Zuckerman (2000). Efficacy of *Bacillus thuringiensis* , *Paecilomycis marquandii* and *Streptomyces costaricanus* with and without organic amendments against *Meloidogyne hapla* infecting lettuce . J. of Nematol., 32: 70-77.

Cotes, A. M.; P. Lepoivre and J. Semal (1996). Correlation between hydrolytic enzyme activities measured in bean seedlings after *Trichoderma koningii* treatment combined with pregermination and the protective effect against *Pythium splendens*. European J. Plant Pathol., 102: 497-506.

DiPietro, A. (1995). Fungal antibioses in biocontrol of plant disease. In:"Allelopathy: Organisms Processes and Applications", Dakshini, 271- 279.

Franklin, M.T.(1949). A cotton blue lactophenol technique for mounting plant parasitic nematodes. J. Helminthol., 23:175-178.

Gadgil, N. J.; H.F.Daginawala; T. Chakrabart and P. Khanna (1995). Enhanced cellulase production by a mutant of *Trichoderma reesei*. Enzyme and Microbiol. Technol., 17: 942-946.

Ghaffar, A. (1995). Biological control of root-rot and root-knot disease complex of vegetableles. Final Research Report, Dept. Botany, University of Karachi, Karachi- Pakistan, 89 pp.

Haggag, W. M. (1998). New approaches for controlling soil borne fungi infecting cucumber plants under greenhouse condition. Ph.D thesis, Ain Shams University, Egypt. PP.180.

Haggag, W. M. and A.W. Amin,(2001). Efficiency of *Trichoderma* species in control of Fusarium-rot, root knot and reniform nematodes disease complex on sunflower. Pakistan J. Biological Sci., 4 : 314-318.

Kanotra, S. and R. S. Mathur (1995). Simple cultural test for relative cellulolytic activity of fungi. Indian J . Phytopathol., 48: 483-488.

Lorito, M.; G. E. Harman;C.K. Hayes; R.M. Broadway; A. Tronsmo; S. L. Woo and A. Di Pietro (1993). Chitinolytic enzymes produced by *Trichoderma harzianum*: antifungal activity of purified endochitinase and chitobiosidase. Phytopathol., 863: 302-307.

Nicola, M. and S. A. George (2000). Hirsutella rhossiliensis and *Verticillium chlamydosporium* as biocontrol agents of the root-knot nematode, *Meloidogyne hapla* on lettuce, J. Nematol. , 32: 85-100.

Parveen, S.; S. Ehieshamul-Haqurand and A. Gaffar (1993). Biological control of *Meloidogyne javanica* on tomato and okra in soil infested with *Fusarium oxysporum*. Pakistan J. Nematol. , 11: 151-156.

Rajeshari, S.; A. Santhi, and C. V . Sivakumar (1998). Effect of organic soil amendments, *Trichoderma viride* Pers. Ex Fr. and carbofuran on the nematode trapping fungi *Arthrobotrys cladodes var. macroides* (Drechsler, 1044) and plant parasitic nematodes. J. Biological Control, 11:49-52.

Reddy, P.P.; M. S. Rao and M. Nagesh (1996). Management of the citrus nematode, *Tylenchulus semipentrans* by integration of *Trichoderma harzianum* with oil cakes. Nematologia Mediterrania, 24: 265-267.

Saifullah ,A.(1996a). Fungal parasitism of young females of *Globodera rostochiensis* and *G. pallida*. Afro-Asian J. Nematol., 6: 17-22.

Saifullah, A. (1996b). Killing potato cyst nematode males: a possible control strategy. Afro-Asian J. Nematol., 6: 23-28.

Siddiqui, I. A.; S. ,Ehteshamul-Haque and A. Ghaffar (1999). Root dip treatment with *Pseudomonas aeruginosa* and *Trichoderma* spp. , in the control of root rot-root knot disease complex in chili. (*Capsicum annum* L.). Pakistan J. Nematol., 17: 67-75.

Spiegel, Y. and I. Chet (1998). Evaluation of *Trichoderma* spp. as a biocontrol agent soil fungi and plant parasitic nematodes in Israel. Integrated Pest Management Reviews, 3:169-175.

Stephan, Z.A.; A.H. EL-Behadli; H.H .AL-Zahroon; B.G. Antoon and S. Sh Georgees (1996). Control of root knot- wilt disease complex on tomato plants. Dirasat In Agricul. Sci., 23: 13-16.

Stephan, Z.A.; I.K. Hassoon and B. G. Antoon (1998). Use of biocontrol agents and nematicides in the control of *Meloidogyne javanica* root-knot nematode on tomato and eggplant. Pakistan J. Nematol., 16: 151-155.

Susan, L.F.M.; N.H. Robin and R. M. Ssayre (1990). Isolation of fungi from *Heterodera glycines* and *in vitro* bioassay for their antagonism to eggs. J. Nematol., 22:532-537.

Wu-Wenshi and W. S. Wu (1998). The effect of bioagent amended potting medium to control *Rhizoctonia solani, Phytophthora capsici* and *Pythium aphanidermatum* for cultivating healthy horticultural crops. Plant Pathol. Bulletin, 7:54-65.

دور فطريات الترايكودرما في مكافحة كل من نيماتودا القطن الكلوية *Rotylenchulus reniformis* و فطر الذبول الفيوزارمي *Fusarium oxysporium* على نباتات عباد الشمس.

أمين وفدى أمين* و وفاء محمد حجاج**

*** قسم الحيوان و النيماتولوجيا الزراعيه – كليه الزراعه – جامعه القاهره – مصر.**

**** قسم أمراض النبات – المركز القومى للبحوث – الدقى – الجيزه – مصر.**

تم دراسه أستخدام خمس من أنواع الفطر ترايكودرما *Trichoderma* spp. كمعلق للجراثيم أو كراشح للفطر في مكافحه نيماتودا القطن الكلويه *Rotylenchulus reniformis* و كذلك مرض الذبول الفيوزارمى المتسبب عن الفطر *Fusarium oxysporium* على نباتات عباد الشمس تحت ظروف البيوت الزراعيه.

تم تنفيذ تجربتين الاولى في المعمل حيث تم تعريض ٢٠٠٠ من الطور المعدى للنيماتودا لمستخلص راشح خمس من فطريات الترايكودرما في أطباق بترى لمده أسبوع حيث سجلت أعداد النيماتودا الحية و الغير نشطه ثم غسلت النيماتودا و تم عدواها على نباتات عباد الشمس لتقييم مدى تطور و تكاثر هذه النيماتودا للافراد الحيه و مدى قدرتها المرضيه مقارنه بالكنترول و هو النيماتودا المعرضه للماء المقطر.

التجربه الثانيه تم فيها دراسه تأثير كل من مستخلص راشح خمس من فطريات الترايكودرما و كذلك معلق جراثيمها على كل من نيماتودا القطن الكلويه و فطر الذبول الفيوزارمى كل على حده أو في شكل عدوى مشتركه كمرض مركب.

تمت الدراسة على نباتات عباد الشمس صنف جيزه ١ تحت ظروف البيوت الزراعيه. و قد دلت النتائج أن استخدام أي من فطريات الترايكودرما الاتيه :- *Trichoderma harzianum, T. viride, T. koningii, T. reesei* or *T. hamatum* قد أعطى نسبه انخفاض معنوي جدا في مكافحة كل من نيماتودا القطن الكلوية و فطر ذبول الفيوزاريم على نباتات عباد الشمس.

و قد دلت النتائج أن أحسن المعاملات كانت كل من فطريات الترايكودرما *Trichoderma harzianum, T. hamatum* and *T. Koningii* عندما أستخدم كل من معلق الجراثيم و كذلك راشح الفطريات حيث أنخفضت أعداد أناث النيماتودا على الجذور و كذلك نسبه و تركير مرض الذبول الفيوزارمى. و كذلك أعطت أحسن النتائج في المكافحة سواء عوملت النباتات بالترايكودرما منفرده او مصاحبه لفطر الفيوزاريوم.

و قد اظهرت النتائج أن أعراض الذبول الفيوزارمى كانت واضحة جدا و مؤثرة عندما صاحب الفطر و جود النيماتودا على نفس النبات مقارنه بالنباتات المعداه بفطر الذبول فقط.

و قد وضح عند أستخدام الترايكودرما تثبيط واضح التأثير على الاعراض الظاهريه للمرض و كذلك نمو الفطر و كذلك خفضت أعداد النيماتودا على النباتات و أنعكس ذلك معنويا على مقاييس نمو نباتات عباد الشمس.

و يمكن تلخيص دور التريكودرما في مكافحه النيماتودا و كذلك مرض الذبول الفيوزارمى للتأثير المباشر لنواتج الأفرازات السامة للنيماتودا وكذلك الفطريات المرضية و إلى تأثيره على المسبب المرضى مباشرة بالتطفل مما يتبع ذلك زيادة تحسين نمو النبات