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## Efficiency of *Trichoderma* Species in Control of Fusarium-Rot, Root Knot and Reniform Nematode Disease Complex on Sunflower

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**Abstract:** The effect of *Trichoderma* species in control of root-rot fungus, *Fusarium solani*, root-knot nematode, *Meloidogyne javanica* or reniform nematode, *Rotylenchulus reniformis* disease complex and on growth of sunflower plant was studied under greenhouse conditions. Treating two weeks old sunflower seedlings cv. Giza 1 with *Trichoderma harzianum*, *T. viride*, *T. koningii*, *T. reesei* or *T. hamatum* gave highly significant effect in control of fusarium-rot disease incidence and nematodes infection on sunflower roots. Infection of *F. solani* was highly increased in *M. javanica* infested soil than *R. reniformis*. Treatments of *Trichoderma* species led to decreasing *Fusarium* cfu counts in soil, infested with either *M. javanica* or *R. reniformis* and also significantly improved the plant growth parameters. *T. hamatum*, *T. harzianum* and *T. koningii* gave the greatest reduction in disease incidence caused by *Fusarium*, *M. javanica* or *R. reniformis* infestation. Generally, there was highly significant reduction in the number of fusarium-wilt disease and nematode population and increases in plant growth parameter of sunflower when treated with *Trichoderma* species.

**Key words:** *Fusarium solani*, *Meloidogyne javanica*, nematodes, root-rot disease, *Rotylenchulus reniformis*, sunflower, *Trichoderma*.

### Introduction

Sunflower plants (*Helianthus annuus* L.) are attacked by a number of infection microorganisms mostly fungi, bacteria and nematodes leading to reduction in yield quantity and quality (Ara *et al.*, 1996; Bhutta *et al.*, 1997 and Amin and Youssef, 1997).

Root-rot diseases still are the most important in affecting sunflowers. *Fusarium solani* is the main pathogen of these diseases (Bhutta *et al.*, 1997). Fusarium-nematode interactions are known to decrease the quantity and quality of major world crops including tomato (Parveen *et al.*, 1993 and Stephan *et al.*, 1996), cotton (Colyer *et al.*, 1997), vegetables (Ghaffar, 1995) and soybean (Mousa, 1994). Because of hazards involved in the use of pesticides, biological control of plant disease has received increasing attention as a promising supplement and now capturing the imagination of plant parasitic nematodes and plant pathogen (Amin, 1999 and Di Pietro, 1995). Of the various bio-agents, fungi of *Trichoderma* species have been known to suppress many soil borne fungi and nematode diseases under greenhouse and field conditions. *T. harzianum*, *T. hamatum* has been found to antagonize fungal plant pathogen and parasitic nematodes (Siddiqi *et al.*, 1999).

The present study aimed to know the effect of *Trichoderma* species in control of *F. solani*, *M. javanica* or *R. reniformis* disease complex and on plant growth of sunflower seedlings.

### Materials and Methods

The fungus, *Fusarium solani* (M.) was isolated from diseased sunflower plants and cultured on Potato Dextrose Agar (PDA). Spore suspension of the fungi was harvested and adjusted to  $3 \times 10^3$  spores/ml. Seeds of sunflower (*Helianthus annuus* L.) cv. Giza 1 were sown in 15 cm diam. pots filled with one kg sandy loam soil (1:1 w/w) free of plant pathogen and parasitic nematodes. After germination (about two weeks), the plants in each pot were thinned to one plant/pot. The pots were divided to two groups, one for root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood, 1949 and the other group for reniform nematode, *Rotylenchulus reniformis* (Linford and Oliviera, 1940). Two days before nematode inoculation,

pots were infested with  $3 \times 10^3$  colony forming units (cfu)  $g^{-1}$  soil of mixed population of *F. solani*, as assessed soil dilution technique. 10 ml of spores' suspension was pipetted around seedling roots in pots. Each pot was inoculated with 2000 infective stages of either *M. javanica* (eggs and juveniles) or *R. reniformis* (juveniles and un-swollen females). Un-inoculated - untreated four pots served as check plants and other four pots inoculated - untreated served as check nematode. Two days after nematode inoculation seedlings were treated with conidia suspension of *Trichoderma harzianum* (Raifi), *T. viride* (Pers.), *T. koningii* (qudem), *T. reesei* (Simmons) and *T. hamatum* (Bon.) at the rate of 10 ml of  $5 \times 10^5$  cfu/g soil per seedling. The pots were arranged in a completely randomized design in green house at  $30 \pm 5$  °C and were watered daily. After nine weeks of nematode inoculation, sunflower plants were carefully uprooted and nematodes in roots were counted (Franklin, 1949). Disease incidence caused by *F. solani* during growth period was recorded. The incidence of *Fusarium* pathogen and *Trichoderma* species in soil rhizosphere was recorded as cfu/g soil and population counts respectively. The number of galls, females and egg-masses as compared to untreated pots was calculated for root-knot nematode and the number of females and egg-masses for *R. reniformis*. Length and weight of shoots and roots and flowering disc weight were recorded. Data was statistically analyzed using New Least Significant Difference (New LSD).

### Results

The effect of *Trichoderma* species on control of fusarium-rot in infested soil with nematodes on sunflower data in (Table 1) indicated that, percentages of root-rot disease caused by *F. solani* alone were 48%, while it was 88 and 68.6% in case of presence of *M. javanica* or *R. reniformis* respectively after 9 weeks of inoculation. Data also showed that, application of antagonistic microorganisms *Trichoderma* species resulted in decrease of *Fusarium* infection. A complete control of fusarium root-rot infection was observed in case of *T. hamatum* treatment. *T. harzianum* was highly effective in controlling fusarium root-rot, while *T. viride* and *T. reesei* were less effective. Data also showed that, the effect of

Haggag and Amin: *Fusarium solani*, *Meloidogyne javanica*, nematodes, root-rot disease, *Rotylenchulus reniformis*

Table 1: Effect of *Trichoderma* species on sunflower in control of fusarium root-rot in infested soil with *Meloidogyne javanica* and *Rotylenchulus reniformis*

Treatment	<i>Meloidogyne javanica</i>				<i>Rotylenchulus reniformis</i>			
	Fusarium disease incidence (Weeks after treatment)							
	2	4	6	9	2	4	6	9
<i>Fusarium solani</i> (F.s)	16.6	24.0	40.0	48.0	16.6	24.0	40.0	48.0
Nematode + <i>Fusarium solani</i>	28.3	49.0	53.3	88.0	18.4	31.6	48.6	68.6
<i>Trichoderma harzianum</i> (T.h.)+(F.s)	0.0	2.4	3.6	5.4	0.0	2.4	3.6	5.4
<i>Trichoderma viride</i> (T.v.)+(F.s)	3.6	5.4	6.3	9.6	3.6	5.4	6.3	9.6
<i>Trichoderma koningii</i> (T.k)+(F.s)	2.3	4.6	6.2	6.2	2.3	4.6	6.2	6.2
<i>Trichoderma reesei</i> (T.r)+(F.s)	4.3	6.6	8.6	11.4	4.3	6.6	8.6	11.4
<i>Trichoderma hamatum</i> (T.ha) + (F.s)	0.0	0.3	0.3	0.3	0.0	0.3	0.3	0.3
(T.h) + (F.s) + Nematode	6.0	8.0	9.6	12.4	0.0	0.3	1.3	1.3
(T.v) + (F.s) + Nematode	8.6	9.4	12.3	18.6	2.3	3.3	4.6	5.6
(T.k) + (F.s) + Nematode	7.2	8.0	11.2	14.7	1.6	2.3	2.3	3.3
(T.r) + (F.s) + Nematode	9.3	11.6	14.6	20.4	3.3	5.6	6.3	9.6
(T.ha) + (F.s) + Nematode	5.3	3.4	6.6	8.4	1.3	1.6	2.3	2.3
New L.S.D 0.5	0.9	1.3	1.6	3.6	1.2	1.9	2.3	3.0
New L.S.D 0.1	1.4	3.3	2.9	6.3	2.9	3.3	4.2	5.6

Table 2: Effect of *Trichoderma* species on sunflower in control of *Meloidogyne javanica* and *Rotylenchulus reniformis* in infested soil with *Fusarium solani*

Treatment	<i>Meloidogyne javanica</i>					<i>Rotylenchulus reniformis</i>		
	Number of							
	Galls	Immature Stages	Female	Egg -mass	% female Reduction	Females	Egg-mass	% female Reduction
Check Nematode (N)	78	41	86	66	0.0	115	84	0.0
Nematode (N) + <i>Fusarium solani</i>	41	23	34	31	55.1	65	61	43.5
<i>Trichoderma harzianum</i> (T.h.) + (N)	41	24	39	28	50.4	10	8	90.5
<i>Trichoderma viride</i> (T.v.) + (N)	64	27	62	46	29.9	35	22	73.8
<i>Trichoderma koningii</i> (T.k) + (N)	48	30	33	17	50.4	41	21	75.0
<i>Trichoderma reesei</i> (T.r) + (N)	47	31	40	35	44.1	62	48	42.9
<i>Trichoderma hamatum</i> (T.ha) + (N)	32	20	26	25	63.8	38	24	71.4
(T.h) + <i>Fusarium solani</i> (F.s) + (N)	54	22	53	44	40.9	61	51	39.3
(T.v) + (F.s) + (N)	28	18	27	19	64.6	71	68	19.0
(T.k) + (F.s) + (N)	30	19	28	21	63.0	74	68	19.0
(T.r) + (F.s) + (N)	58	31	50	45	36.2	71	69	17.9
(T.ha) + (F.s) + (N)	53	29	51	44	37.0	42	38	54.8
New L.S.D 0.5	9	10.3	12.8	6.6	-	12.3	10.5	-
New L.S.D 0.1	11.7	13.4	16.9	8.8	-	15.8	13.5	-

Table 3: Effect of *Trichoderma* species on the growth of *Fusarium solani* in soil rhizosphere of sunflower grown in infested soil with *Meloidogyne javanica* or *Rotylenchulus reniformis*

Treatment	<i>Meloidogyne javanica</i>				<i>Rotylenchulus reniformis</i>			
	Cfu * 10 <sup>3</sup> /Weeks after treatment							
	1	3	6	9	1	3	6	9
<i>Fusarium solani</i> (F.s)	3.3	5.2	6.6	9.4	3.3	5.2	6.6	9.4
Nematode + <i>Fusarium solani</i>	4.3	9.2	14.6	24.4	3.8	5.3	11.2	18.6
<i>Trichoderma harzianum</i> (T.h.) + (F.s)	0.3	0.6	1.6	1.3	0.3	0.6	1.6	1.3
<i>Trichoderma viride</i> (T.v.) + (F.s)	1.3	2.6	2.3	2.8	1.3	2.6	2.3	2.8
<i>Trichoderma koningii</i> (T.k) + (F.s)	0.6	1.3	1.8	1.5	0.6	1.3	1.8	1.5
<i>Trichoderma reesei</i> (T.r) + (F.s)	1.6	2.8	2.6	3.3	1.6	2.8	2.6	3.3
<i>Trichoderma hamatum</i> (T.ha) + (F.s)	0.3	0.3	0.6	0.6	0.3	0.3	0.6	0.6
(T.h)+(F.s) + Nematode	0.6	1.3	1.6	2.3	0.3	1.0	1.3	1.6
(T.v) + (F.s) + Nematode	2.3	3.0	3.3	3.6	2.0	2.8	3.0	3.3
(T.k) + (F.s) + Nematode	1.6	2.6	2.8	2.6	1.3	1.6	2.3	2.3
(T.r) + (F.s) + Nematode	2.6	3.3	3.6	4.2	2.3	3.3	3.0	3.6
(T.ha) + (F.s) + Nematode	0.6	1.0	1.3	1.3	0.6	0.6	1.0	1.0
New L.S.D 0.5	1.3	1.6	1.8	2.2	1.2	1.4	1.7	2.0
New L.S.D 0.1	2.6	2.9	3.3	5.6	2.4	2.6	3.0	4.9

\* colony forming units

*Trichoderma* species on fungi-nematodes interaction. It means that the percentage of root-rot was greatly decreased in soil infested with either *M. javanica* or *R. reniformis*. *T. hamatum*

and *T. harzianum* showed more effect in reducing disease incidence in the soil infested with *M. javanica* or *R. reniformis* compared to *T. reesei*.

Haggag and Amin: *Fusarium solani*, *Meloidogyne javanica*, nematodes, root-rot disease, *Rotylenchulus reniformis*

Table 4: Population counts of *Trichoderma* species in soil rhizosphere of sunflower grown in infested soil with *Fusarium solani*, *Meloidogyne javanica* and *Rotylenchulus reniformis*.

Treatment	<i>Meloidogyne javanica</i>				<i>Rotylenchulus reniformis</i>			
	Population counts (cfu *10 <sup>4</sup> / Weeks after treatment				Population counts (cfu *10 <sup>4</sup> / Weeks after treatment			
	2wvk	4wvk	6wvk	9wvk	2wvk	4wvk	6wvk	9wvk
<i>Trichoderma harzianum</i> (T.h.)	4.6	8.4	14.6	22.3	4.6	8.4	14.6	22.3
<i>Trichoderma viride</i> (T.v.)	2.6	5.6	7.5	9.6	2.6	5.6	7.5	9.6
<i>Trichoderma koningii</i> (T.k)	3.6	7.8	9.1	11.6	3.6	7.8	9.1	11.6
<i>Trichoderma reesei</i> (T.r)	3.0	4.2	5.2	7.6	3.0	4.2	5.2	7.6
<i>Trichoderma hamatum</i> (T.ha)	8.6	10.4	16.4	32.3	8.6	10.4	16.4	32.3
(T.h.) + <i>Fusarium solani</i> (F.s)	5.9	9.5	16.6	28.6	5.9	9.5	16.6	28.6
(T.v.) + (F.s)	3.8	6.8	9.4	13.6	3.8	6.8	9.4	13.6
(T.k) + (F.s)	3.6	8.6	11.1	15.6	5.6	8.6	11.1	15.6
(T.r) + (F.s)	4.3	7.2	8.6	10.4	4.3	7.2	8.6	10.4
(T.ha) + (F.s)	9.5	14.6	22.6	48.6	9.5	14.6	22.6	48.6
(T.h) + Nematode (N)	11.6	18.6	24.3	31.6	9.3	13.4	21.6	23.7
(T.v) + Nematode	8.6	14.6	20.4	26.7	6.7	10.4	16.4	18.2
(T.k) + Nematode	9.7	16.4	18.6	26.0	7.1	12.1	14.4	20.2
(T.r) + Nematode	6.2	7.6	9.4	14.6	4.6	5.4	6.4	11.7
(T.ha) + Nematode	16.6	20.4	36.6	56.6	11.4	17.8	24.6	48.8
(T.h) + (F.s) + (N)	12.4	22.6	34.0	41.6	10.6	18.6	29.0	29.9
(T.v) + (F.s) + (N)	10.6	16.4	21.3	34.2	8.0	15.0	21.4	24.6
(T.k) + (F.s) + (N)	14.6	18.7	26.6	30.3	9.0	16.4	28.0	30.1
(T.r) + (F.s) + (N)	7.6	8.6	10.8	18.6	5.1	6.8	10.6	14.6
(T.ha) + (F.s) + (N)	19.1	26.0	41.6	64.0	16.4	23.0	37.0	53.6
New L.S.D 0.5	2.6	3.5	9.4	13.6	2.3	3.0	7.2	11.4
New L.S.D 0.1	5.8	6.8	12.4	16.5	5.1	5.8	10.3	15.7

\* colony forming unit

Table 5: Effect of *Trichoderma* species on the growth of sunflower in infested soil with *Fusarium solani* and or *Rotylenchulus reniformis*.

Treatment	Plant length (cm)		Plant fresh weight (g)		Plant dray weight (g)		Flower disc weight (g)	
	Root	Shoot	Root	Shoot	Root	Shoot	Fresh	Dray
<i>Trichoderma harzianum</i> (T.h.)	13.6	84.1	3.9	11.5	0.6	3.0	1.6	0.4
<i>Trichoderma viride</i> (T.v.)	12.5	80.0	2.0	10.6	0.5	2.8	1.2	1.4
<i>Trichoderma koningii</i> (T.k)	13.1	81.2	3.2	10.8	0.5	2.9	1.3	0.4
<i>Trichoderma reesei</i> (T.r)	12.9	76.2	3.0	9.0	0.4	2.7	1.3	0.4
<i>Trichoderma hamatum</i> (T.ha)	14.0	85.0	3.7	11.5	0.6	3.3	1.6	0.4
(T.h.) + <i>Fusarium solani</i> (F.s)	11.9	80.6	3.5	10.9	0.9	2.8	1.3	0.4
(T.v.) + (F.s)	10.9	70.4	2.4	8.2	0.4	2.4	1.1	0.3
(T.k) + (F.s)	11.2	75.0	3.1	8.4	0.4	2.6	1.1	0.3
(T.r) + (F.s)	10.0	69.5	3.0	7.0	0.4	2.0	1.0	0.3
(T.ha) + (F.s)	11.8	81.3	3.4	10.8	0.4	2.8	1.4	0.5
(T.h) + <i>Rotylenchulus reniformis</i> (R.r)	12.9	83.3	3.5	11.2	0.5	2.9	1.3	0.4
(T.v) + (R.r)	12.0	79.4	2.9	11.4	0.4	2.7	1.1	0.3
(T.k) + (R.r)	12.6	82.1	3.6	10.7	0.5	2.8	1.3	0.4
(T.r) + (R.r)	11.4	71.6	2.8	9.5	0.4	2.4	1.0	0.4
(T.ha) + (R.r)	12.3	86.6	3.9	11.0	0.5	2.9	1.5	0.4
(T.h) + (F.s) + (R.r)	12.3	81.6	3.2	10.0	0.5	2.8	1.3	0.4
(T.v) + (F.s) + (R.r)	11.6	79.4	2.7	7.9	0.4	1.9	1.0	0.3
(T.k) + (F.s) + (R.r)	12.6	80.0	3.3	8.0	0.4	2.5	1.2	0.3
(T.r) + (F.s) + (R.r)	11.0	74.0	2.6	7.0	0.4	1.7	1.2	0.2
(T.ha) + (F.s) + (R.r)	12.9	82.9	3.2	10.2	0.5	2.4	1.3	0.4
Check ( <i>Fusarium solani</i> )	6.4	58.0	0.9	7.1	0.1	1.6	0.4	0.2
Check ( <i>Rotylenchulus reniformis</i> (R.r))	5.6	55.0	0.8	5.0	0.1	1.0	0.4	0.2
Check (F.s.) + <i>R. reniformis</i> (R.r)	5.2	51.2	0.7	3.9	0.7	0.1	0.3	0.1
Check (plant)	7.8	81.3	1.0	7.6	0.3	2.0	0.6	0.3
New L.S.D 0.5	0.52	3.3	0.3	0.52	0.16	0.10	0.21	0.12
New L.S.D 0.1	2.2	5.7	0.78	1.2	0.5	0.39	0.5	0.36

Use of *Trichoderma* species alone or in combination with *F. solani* gave a highly significant ( $P < 0.01$ ) reduction of females and egg-masses of root-knot and reniform nematode (Table 2). Great reduction in root-knot nematode females (more than 50%) was recorded where *T. hamatum* (63.8%), *T. harzianum* (50.4%) and *T. koningii* (50.4%) were used alone followed by *T. viride* (64.6%) and *T. koningii* (63%) when treated in combination with *F. solani*. The same trend was noticed in the number of galls and immature stages in roots. Highly significant reductions were observed on female

reduction of *R. reniformis* (Table 2) where *T. harzianum* (90.5%) and *T. koningii* (75%) were used followed by *T. viride* (73.8%) and *T. hamatum* (71.4%) when used alone. Whereas, *Trichoderma* species treated with *F. solani*, a highly significant reduction in reniform nematode, was observed in case of *T. hamatum* (54.8%) and *T. harzianum* (39.3%). It also noticed that, use of *F. solani* with either *M. javanica* or *R. reniformis* gave 55.1 and 43.5% female reduction respectively. Due to effect of *Trichoderma* species on *Fusarium* population treated with *M. javanica* or *R. reniformis* infested

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Table 6: Effect of *Trichoderma* species on growth of sunflower in infested soil with *Fusarium solani* and *Meloidogyne javanica*.

Treatment	Plant length (cm)		Plant fresh weight (g)		Plant dry weight (g)		Flower disc weight (g)	
	Root	Shoot	Root	Shoot	Root	Shoot	Fresh	Dray
<i>Trichoderma harzianum</i> (T.h.)	12.8	86.3	3.9	12.3	0.6	2.5	1.4	0.5
<i>Trichoderma viride</i> (T.v.)	12.9	83.3	2.7	10.1	0.5	1.9	1.2	0.4
<i>Trichoderma koningii</i> (T.k)	12.5	80.5	3.3	10.6	0.6	1.9	1.3	0.4
<i>Trichoderma reesei</i> (T.r)	13.0	86.5	2.6	10.2	0.5	1.7	1.2	0.4
<i>Trichoderma hamatum</i> (T.ha)	11.8	88.0	3.8	11.6	0.7	2.4	1.6	0.4
(T.h.) + ( <i>Fusarium solani</i> (F.s)	12.8	82.6	3.6	11.6	0.4	2.6	1.3	0.4
(T.v.) + (F.s)	12.3	73.6	2.3	7.8	0.4	2.5	1.1	0.4
(T.k) + (F.s)	11.6	71.6	3.3	8.0	0.4	2.4	1.2	0.4
(T.r) + (F.s)	10.8	70.0	3.0	7.3	0.5	1.9	1.1	0.4
(T.ha) + (F.s)	12.3	86.0	3.5	9.8	0.4	2.8	1.6	0.5
(T.h) + <i>Meloidogyne javanica</i> (M.j)	12.6	85.5	3.2	10.6	0.4	2.7	1.4	0.4
(T.v) + (M.j)	12.0	76.6	2.6	10.2	0.4	2.6	1.1	0.4
(T.k) + (M.j)	12.5	80.0	3.0	10.5	0.4	2.1	1.4	0.4
(T.r) + (M.j)	11.0	74.0	2.3	8.8	0.5	2.0	1.0	0.3
(T.ha) + (M.j)	12.3	84.3	3.3	10.6	0.4	2.1	1.7	0.5
(T.h) + (F.s) + (M.j)	11.9	79.8	3.0	9.1	0.4	2.0	1.2	0.4
(Tv) + (F.s) + (M.j)	11.6	77.3	2.2	7.1	0.4	1.7	0.8	0.3
(T.k) + (F.s) + (M.j)	11.3	75.5	2.8	7.4	0.4	2.0	1.1	0.3
(T.r) + (F.s) + (M.j)	10.2	70.1	2.1	7.0	0.3	1.9	0.8	0.2
(T.ha) + (F.s) + (M.j)	12.0	80.7	3.0	9.2	0.4	2.2	1.2	0.3
Check ( <i>F. solani</i> )	6.2	59.3	0.9	7.0	0.2	1.5	0.7	0.2
Check ( <i>M. javanica</i> (M.j)).	12.3	71.0	3.1	3.3	0.8	0.5	0.1	0.0
Check (F.s.) + Nematode (M.j)	4.6	45.6	3.7	2.8	0.9	0.2	0.0	0.5
Check (plant)	8.0	80.0	1.2	8.0	0.3	2.0	0.7	0.4
New L.S.D 0.5	0.5	4.3	0.4	0.8	0.13	0.2	0.13	0.1
New L.S.D 0.1	1.2	6.6	0.9	2.1	0.43	0.48	0.46	0.30

soil. Population density of *F. solani* was increased in soil rhizosphere until 9 weeks of plant growth by  $9.4 \times 10^4$  cfu/g soil (Table 3). Data also showed that population density of *F. solani* was greatly increased in soil infested with either *M. javanica* or *R. reniformis*. Inoculation of soil with *M. javanica*, increased the *F. solani* population counts than did reniform nematode, *R. reniformis*. Treatment of *Trichoderma* species recorded minimum population density of *F. solani*. *T. hamatum* and *T. harzianum* gave high reduction in *F. solani* by  $0.6 \times 10^4$  and  $1.3 \times 10^4$  cfu/g soil respectively. Moreover, these antagonisms gave great effect in reducing *Fusarium* count in infested soil with either *M. javanica* or *R. reniformis*. Concerning *Trichoderma* population in fungal-nematodes infested soil, data indicated that population density of *Trichoderma* species was increased in soil rhizosphere until 9 weeks of plant growth (Table 4). *T. hamatum* and *T. harzianum* counts were higher in soil rhizosphere during 9 weeks in comparison with other *Trichoderma* species. Moreover, *T. koningii* colonization was multiplied three times from one to 9 weeks in soil rhizosphere. The infested soil with both *Fusarium* and nematodes, the result, indicated increased population of *Trichoderma* in soil rhizosphere. The highest population count was obtained in infested soil with *M. javanica* alone or with *F. solani*. Also, the addition of *T. harzianum* or *T. hamatum* in infested soil with *F. solani* and *M. javanica* or *R. reniformis* yielded the highest population in soil rhizosphere.

Due to the effectiveness of *Trichoderma* species on plant growth in infested soil with fungal-nematode disease complex, results present in Tables 5 and 6 revealed that, soil infested with *F. solani* significantly ( $p < 0.05$ ) decreased plant height and weight. Also, soil infested with *F. solani* when mixed with *M. javanica* led to lowest value of plant length, weight and flower disc weight. But the treatment of *Trichoderma* species alone or in combination with root-rot fungus or nematodes, significantly increased plant growth parameters. *T. hamatum*, *T. harzianum* and *T. koningii* gave the best result in plant growth parameter. While, a slight increase was observed in *T. reesei*.

## Discussion

In the present study, data showed that *F. solani* treated with root-knot or reniform nematodes increased root-rot disease incidence to a great extent, than plant infested with the pathogen alone (Table 1). Also, plant infection was greatly increased in soil infested with *M. javanica* than *R. reniformis*. This result can be explained that nematodes predispose the plant pathogen invasion, as has already been reported (Stephan *et al.*, 1996 and Siddiqui *et al.*, 1999). The interaction between the pathogen fungi and the endo-parasitic nematode, *M. javanica* gave a great damage to plant growth than the semi-endo parasite nematode, *R. reniformis* (Mousa, 1994). The addition of *Trichoderma* species in controlling disease complex caused by root-rot and root-knot or reniform nematodes, data indicated that, significant reduction in the pathogen and nematode population and increased plant growth parameter. Several species of *Trichoderma* have been reported to suppress soil borne disease fungi including *Fusarium* and parasitic nematodes (Siddiqui *et al.*, 1999). *Trichoderma* species are known to produce other secondary metabolites such as enzymes (Di Pietro, 1995). These enzymes in biocontrol can be assigned both mechanisms, parasitism and antibiosis. In particular cell wall degrading enzymes such as chitinase, B-1,3-glucanases and cellulase; but only important features of micoparasites for colonization of their pathogens, but exhibit considerable antifungal activity on their one. Chitinase enzymes have been considered important in the biocontrol pathogenic fungi at low concentration because of their ability to degrade fungal cell walls, of which a major component is chitin (Lorito *et al.*, 1993). Also, most plant parasitic nematode eggs have a second layer is a thick chitin layer of eggshell, which is secreted by the egg (Lee, 1965). Recently, has been achieved for various chitinases, B-1, B-glucanase and cellulase from biocontrol fungi, *T. harzianum*, *T. hamatum* and *T. koningii* (Belanger *et al.*, 1995 and Cotes *et al.*, 1996). Whereas cellulase, glucanase and glucosidase are main enzymes produced by *T. viride* and *T. reesei* (Gadgil *et al.*, 1995 and

Kanotra and Mathur, 1995). Lorito *et al.* (1993) found that two purified chitinase from *T. harzianum* inhibited *F. solani* growth at 30 g/ml concentration. Besides parasitism of root-knot nematode it also hypothesized that the production of nematicidal compounds by *Trichoderma* species directly affected the nematode or made rootless attractive which might have resulted the reduction in the nematode population. These results confirm the report of Reddy *et al.* (1996) and Siddiqui *et al.* (1999). Moreover, many authors recorded that, *Trichoderma* species as nematophagous fungi on eggs, larvae and females of cyst nematodes (Susan *et al.*, 1990) In this study, treatment of *Trichoderma* species successfully reduced fungal-nematode disease complex and increased plant growth parameters. In conclusion, using biocontrol agents in reducing the damages of fungi-nematodes disease complex in order to manage the disease on economic crops is necessary.

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