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Elucidating the Parasitic Capabilities of *Trichoderma* against *Meloidogyne javanica* on Tomato

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Abstract: The optimal conditions are require to market *Trichoderma* as a biocontrol agent against soilborne fungi and nematodes. These include a proper formulation, an efficient delivery system and alternative methods for application of *Trichoderma*. In this research, three factors of time were spoted to inoculate in complete randomized design with five repeats for each treatment. In first time factor, *Trichoderma* fungi and 2000 nematode larvaes was inoculated to the soil so that this soil had been mixed with peat and bran wheat ratio 1% and tomato three-week-seedlings were transmitted to the pots after 18 days. In second time factor, the mixture of mentioned soil was inoculated with fungi and nematode in time of transmission of three-week-seedlings. In third time factor, *Trichoderma* fungi were inoculated to the mixture of mentioned soil and seedlings were transmitted and 2000 larvaes were inoculated to the soil after 18 days. Disease and growth indices were measured after 45 days. First time factor gave the best control and second time factor gave the worst control. Roots dry weight and plant aerial organs were increased compared to testimonial infected plants, followed by 22-72% and 19-51%, respectively. Galls diameter, nematode penetration and nematode population were reduced compared to testimonial infected plants, followed by 21-70%, 19-72%, 23-68% and 28-69%, respectively. These differences were significant compared to contaminated control in level of five percentages. When nematode exposures against *Trichoderma* fungi more than 10 days, paralysis in acidic productions aggregation and drop behind to receive to the root and roots protect against nematode attack.

Key words: Gall, root-knot nematode, time factor, tomato, *Trichoderma*

INTRODUCTION

Plant-parasitic nematodes cause great economic losses to agricultural crops in world wide. Root-knot nematodes (RKNs, *Meloidogyne* spp.) are sedentary, polyphagous root endoparasites. Species such as *M. javanica* and *M. incognita* are among the major limiting factors in the production of field and plantation crops (Sharon *et al*., 2007). *Meloidogyne* spp. is able to infect more than 2000 plant species (Jung and Wyss, 1999). Studies have shown that root-knot nematodes can cause suppression in yield of tomato as high as 85% (Sasser, 1979; Taylor and Sasser, 1978).

Biological control of soil borne plant pathogens by antagonistic microorganisms is a potential nonchemical means of plant-disease control. One of those organisms, *Trichoderma* spp., which is an active mycoparasite, has been considered as biocontrol agent of foliar (Elad *et al*., 1995) and soil borne diseases (Papavizas, 1985) and plant-parasitic soilborne nematodes (Spiegel and Chet, 1998). *Trichoderma* spp. has also been described as biocontrol agents against plant-parasitic nematodes. Several reports showed that *Trichoderma* spp. is able to suppress *Meloidogyne* spp. populations (one of the most economically nematodes worldwide) and increase crop yields (Rao *et al*., 1998; Sharon *et al*., 2001; Spiegel and Chet, 1998; Windham *et al*., 1989). Although the information about the mechanisms of this fungal activity against root-knot nematodes is limited, the ability of *T. harzianum* Rifai to colonize eggs and infect second stage juveniles (J2) *in vitro* has been demonstrated (Saifullah and Thomas, 1996; Sharon *et al*., 2001). The strong biodegradation and substrate-colonization properties of many *Trichoderma* strains are the result of an amazing metabolic versatility and a high secretory potential that lead to the production of diversified sets of hydrolytic enzymes. A principal role in mycoparasitism has been attributed to chitinases and glucanases. However, fungal proteases may be significantly involved in antagonistic activity, not only in the breakdown of the

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host cell wall (composed of chitin and glucan polymers embedded in and covalently linked to, a protein matrix, but also by acting as proteolytic inactivators of pathogen enzymes involved in the plant infection process (Elad and Kapat, 1999). Extracellular proteolytic activities in *Trichoderma* species have long been recognized and they have been attributed to antagonistic and biocontrol activities (Elad and Kapat, 1999).

All *Trichoderma* isolates exhibited nematode biocontrol activity in pot experiments with tomato plants. Parasitic interactions were demonstrated in planta: females and egg masses dissected from tomato roots grown in *T. asperellum*-203-treated soil were examined and found to be parasitized by the fungus (Sharon *et al.*, 2007).

Various mechanisms have been suggested for the biocontrol activity of *Trichoderma* against phytopathogenic fungi: antibiosis, competition, enzymatic hydrolysis, parasitism and systemic induced resistance (Chet *et al.*, 1997; Harman *et al.*, 2004). Several attempts have been made to use *Trichoderma* as a biocontrol agent against plant-parasitic nematodes (Windham *et al.*, 1989; Rao *et al.*, 1998). Direct interactions between *T. harzianum* and the potato cyst nematode *Globodera rostochiensis* have been demonstrated *in vitro* by Saifullah and Thomas (1996). Biocontrol activities of *T. asperellum*-203 and *T. atroviride* IMI 206040 (both fungi were previously defined as strains of *T. harzianum*) have been reported against *M. javanica* in soil (Sharon *et al.*, 2001). In addition, the protease encoded by prb1 also appears to participate in virulence against the nematode *Meloidogyne javanica* (Sharon *et al.*, 2001).

This study demonstrates biocontrol activities of *Trichoderma* isolates and their parasitic capabilities on *M. javanica*, elucidate the importance of the proper time factor to inoculate and the gelatinous matrix in the fungal parasitism.

MATERIALS AND METHODS

Separation, identification and reproduction of Nematode: Mature females were dissected out from large galls on the roots of tomato plants and perineal patterns slides (10-20) from each sample or locality were prepared and examined under microscope. To study their characteristics, perennial patterns of mature females of root-knot nematode were prepared to identificate. At least, 10 perennial patterns of each sample of nematode species were examined to make more accurate identification (Jepson, 1987). For reproduction of nematode, single egg mass was separated from terminal of the females' body and were inserted to the soil by making three holes around the tomato seedlings (Race of *Early urbana*) in greenhouse conditions.

Nematode inoculum: *Meloidogyne javanica* nematode was obtained from pure cultures maintained on roots of tomato. The entire root system was dipped in water and soil was removed gently without detaching egg sacs. Eggs were extracted by vigorous shaking of infested roots in a 1% sodium hypochlorite solution for 3 min. The resulting suspension was then passed through a range of different mesh-size sieves. The eggs were collected on a fine sieve (38 mm) and washed in tap water to remove all traces of sodium hypochlorite before use. Hatched juveniles of *M. javanica* were obtained by placing the eggs in sterile distilled water for 5 days at 28°C. The inoculum was used in the laboratory and glasshouse tests (Siddiqui *et al.*, 2006).

The evaluation of nematode population in root: The number of juveniles, eggs and females in the roots were also estimated. The roots were cut into small pieces and mixed; 1 g root was macerated for 45 sec in Waring blender to recover nematode eggs, females and larvae. The total root population of nematodes was determined with the number of larvae and females present in 1 g root and by multiplying it with total weight of root (Siddiqui and Mahmood, 1998).

The Larva mortality of nematode in percent: The Larva mortality of nematode in percent was determined in infected roots compared to control roots via Abbott's formula (Abbott, 1925).

The Larva mortality of nematode in percent = $100\times[1-(Tn/Cn)]$

Tn = Nematode population after inoculation
Cn = Nematode population in control
n = Nematode population
T = Treatment (fungi or bacteria)
C = Control (non treatment)

***Trichoderma* inoculum:** Two *Trichoderma harzianum* strains (T1 and T2) were used in this work (Table 1). Mention strains were obtained from the Department of Plant Pathology, University of Tehran, Iran. Two strains of *T. harzianum* that had been evaluated for their nematicidal activity *in vitro* before hand, was cultured in Agar water medium. Plates was incubated in 25°C for 5 days. A mixture of peat and wheat bran (1:1, v/v) has been used extensively as a medium to prepare

T. harzianum inoculum (Sivan *et al.*, 1984). In this modified preparation, the pH remained constant and low (5.5) during the entire growth period, thus was prevented bacterial contamination. The mention mixture was autoclaved for 15 min, twice in two days continuously. The fungi suspension (10 mL, 10^8 CFU g^{-1} mL^{-1}) was inserted to sterile mixture in the Erlen. The mention mixture was incubated for 18 days in 25°C (Spiegel and Chet, 1998).

Biocontrol of root-knot nematode by *Trichoderma* (*In vitro*): Parasitism attachment of the *Trichoderma* isolates was bioassayed on various life stages of *M. javanica* in 96-well plates. The plates were contained with 80 mL of diluted medium [20-fold diluted Potato Dextrose Broth (PDB) (DifcoTM); 0.05% w/v KCl; 0.05% w/v $MgSO_4.7H_2O$; 1 mM $CaCl_2$], 10 mL of an aqueous suspension of 10^5 fungal conidia mL^{-1} and about 100 J2s or eggs in 10 mL water, or two egg masses. This diluted medium was designed as a minimal medium to support fungal germination and spores growth, which enabled microscopic observations and fungal growth in control treatments without nematodes (diluted 20-fold PDB (1:20, PDB:Water) was selected for suitable growth after testing several other dilutions between 10 and 30-fold). There were five replicates for each treatment. Control treatments consisted of nematodes without the fungi and/or fungi without the nematodes. Percentages of parasitized nematode eggs and J2s were determined after 48 h, using an inverted microscope. Attachment of fungal conidia to various nematode life stages was observed.

Conidia-agglutination assays: Agglutination assays were performed in round-bottom 96-well plates with gm suspension in serial two fold dilutions. Each wells contained 50 mL of g suspension, 50 mL of conidial suspension and 100 mL PBS pH 7.4 containing 2 mM $CaCl_2$, $MgCl_2$ and $MnCl_2$, or Ca^{2+}- Mg^{2+}- and Mn^{2+}-free PBS, or PBS containing each of the ions separately. Conidial suspensions of T1 or T2. Atroviride contained ca. 10^6 conidial mL^{-1} and were adjusted to obtain clear conidial sediment in the control (Sharon *et al.*, 2007).

Biocontrol of root-knot nematode by *Trichoderma* (In greenhouse): Plants was prepared and cultivated similar to mentioned experiment. After one week, the plants were treated with one of the following fungi. The *Trichoderma* inoculum was mixtured (1% w/w) with soil in each pot (Sharon *et al.*, 2001). Each treatment was replicated 5 times. In this section, three-time-factor was used in consist: In first time factor (t_1), *Trichoderma* fungi and 2000 nematode larvaes was inoculated to the soil so that this soil had been mixed with peat and bran wheat ratio 1% and tomato three-week-seedlings were transmitted to the pots after 18 days. In second time factor (t_2), the mixture of mentioned soil was inoculated with fungi and nematode in time of transmission of three-week-seedlings. In third time factor (t_3), *Trichoderma* fungi were inoculated to the mixture of mentioned soil and seedlings were transmitted and 2000 larvaes were inoculated to the soil after 18 days (Spiegel and Chet, 1998). The plant samples were taken 45 days after nematode inoculation and shoot and root dry weight, number of galls per root system, gall diameter, nematode population in soil and root were measured and recorded.

Statistical analyses: Data were subjected to one-way analysis of variance (ANOVA) followed by the Complete Randomized Design (CRD) test using SAS software (ver. 9.1, USA). Meanwhile each treatment was replicated 5 times.

RESULTS

Biocontrol of root-knot nematode by *Trichoderma* (*In vitro*)

Percentages of parasitized nematode eggs and J2s: Egg and J2 were exposed with *Trichoderma* isolates. Conidium of *T. harzianum* isolates (T1 and T2) adhered to the gm around the egg masses and prolific fungal growth was observed as upon parasitism of the egg (Fig. 1b): germinating hyphae penetrated to the egg masses and parasitized the eggs and J2s within them. Conidial attachment to the gm originated eggs and J2s was observed, followed by direct parasitism of hyphae coiling around the J2s (Fig. 1a) and penetrating them and egg colonization by the fungi Conidia and hyphae were tightly attached to the egg surfaces (Fig. 1b). Variations were observed among the different *Trichoderma* isolates in their attachment and parasitic capabilities (Table 1). *Trichoderma* T2 isolate exhibited conidial adhesion to the eggs less than T1 isolate and the difference was significant in these treatments ($p<0.01$). Also T2 and T1 were highly effective in terms of parasitism on J2s but the difference was not significant in these treatments ($p<0.01$).

Table 1: Mean comparison of Root-Knot nematode control by two strains of *Trichoderma* (*In vitro*)

Trichoderma isolate	Parasitism on eggs (%)	Parasitism on J2s (%)
T1	68.90b	83.87a
T2	88.36a	80.80a
M	7.30b	10.80c

Means with the same letter have no significant difference at 1% level

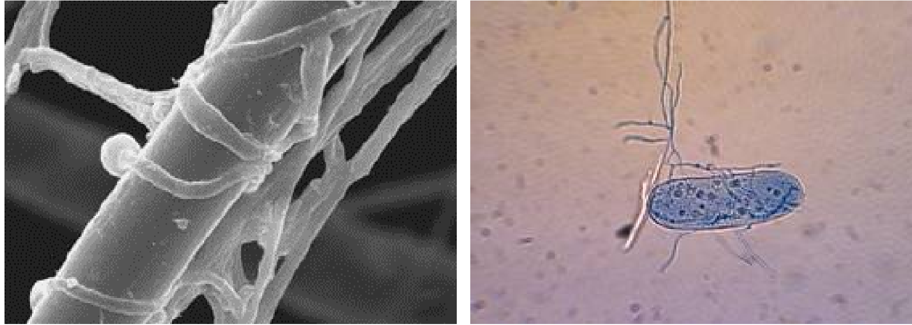

Fig. 1: (a) Scanning electron micrographs of nylon fibers coated with gelatinous matrix (gm). Fungal parasitic-like behaviour: coiling and branching, bar = 20 mm (direct parasitism of hyphae coiling around the Second-stage juvenile of *Meloidogyne javanica* nematode); (b) Egg within the egg mass colonized by the fungus. The fungus was stained with aniline blue (Colonization of egg by the *Trichoderma harzianum* Conidia and hyphae were tightly attached to the egg surfaces) (100x)

Table 2: Mean comparison of Root-Knot nematode control by two strains of *Trichoderma* in greenhouse

Trichoderma strain	Top wet weight (g)	Top dry weight (g)	Root dry weight (g)
T2	19.31a	2.79a	2.00a
T1	16.46b	2.51ab	1.58b
Control	15.32bc	2.29bc	1.51b
$T2Mt_1$	13.36cd	2.16c	1.31bc
$T2Mt_2$	10.62ef	1.49d	0.66d
$T2Mt_3$	12.37de	1.57d	1.08c
$T1Mt_1$	9.75f	2.07c	1.29bc
$T1Mt_2$	4.95gh	1.47d	0.52d
$T1Mt_3$	6.54g	1.69d	1.11c
M	3.92h	1.12e	0.40d

Means with the same letter have no significant difference at 5% level. M is the inoculated plants by *Meloidogyne javanica*. Control is the nontreated plants with nematode or fungi. In first time factor (TMt1), *Trichoderma* fungi and 2000 nematode larvaes was inoculated to the soil so that this soil had been mixed with peat and bran wheat ratio 1% and tomato three-week-seedlings were transmitted to the pots. In second time factor (TMt2), the soil was inoculated with fungi and nematode in time of transmission of three-week- seedlings. In third time factor (TMt3), *Trichoderma* fungi were inoculated to the soil and seedlings were transmitted and 2000 larvaes were inoculated to the soil after 12 days

Percentages of parasitized nematode eggs in T1 and T2, followed by 69 and 80%, respectively (Table 1). Percentages of parasitized nematode J2s in T1 and T2, followed by 84 and 88%, respectively (Table 1).

Biocontrol of root-knot nematode by *Trichoderma* (in greenhouse): In infected plants to nematode and *Trichoderma* had significant differences in the growth and disease indices compared to nematode-infected nontreated plants (Table 2).

Growth indices: T2 isolate was the most effective in increase of the top fresh weight of the plants ($p<0.05$). Top fresh weight of the plants was in range of 8 to 21%, respectively (Table 2). In infected plants with nematode and *Trichoderma,* increase of the top fresh weight was in range of 21 to 71%, respectively (Table 2). Their difference was significant compared to nematode-infected nontreated plants ($p<0.05$). $T2Mt_1$ treatment had the most increase in the top fresh weight and $T1Mt_2$ treatment had the least increase in the top fresh weight.

The increase of the top fresh weight in infected plants with T1 and T2 isolates was in range of 6 to 25%, respectively. In nematode-infected plants and *Trichoderma,* increase of the top dry weight of the plants was in range of 22 to 70%, respectively (Table 2). $T2Mt_1$ treatment had the most increase in the top dry weight and $T1Mt_2$ treatment had the least increase in the top dry weight. The effect of T2Mt1 treatment was significant compared to nematode-infected nontreated plants but the effect of $T2Mt_2$ treatment was not significant compared to nematode-infected nontreated plants ($p<0.05$).

Damage to the nematode-infected plants was 73% compared to health nontreated plants. The effect of T2 and T1 isolates in increase of the root dry weight was significant compared to nematode-infected nontreated plants ($p<0.05$). The effect of T2 treatment was more than T1 isolates. The effect of T2 and T1 isolates in increase of the root dry weight, followed by 18 and 8%, respectively (Table 2). The root dry weight in the plants of treated with *Trichoderma* and nematode was in range of 19 to 52% compared to nematode-infected nontreated plants (Table 2).

$T2Mt_1$ treatment had the most increase in the root dry weight and $T1Mt_2$ treatment had the least increase in the root dry weight. The effect of these treatments was significant compared to nematode-infected plants ($p<0.05$). Damage to nematode-infected nontreated plants was 52% compared to health nontreated plants (Table 2).

Table 3: Mean comparison of Root-Knot nematode control by two strains of *Trichoderma* (In greenhouse)

Trichoderma strain	Gall number	Gall diameter (mm)	Nematode penetration in root	Nematode population in soil
M	293a	7.12a	572a	2285a
$T1Mt_1$	106e	2.98e	222d	808c
$T1Mt_2$	237b	5.70b	447b	1041c
$T1Mt_3$	197c	3.70d	325c	1665b
$T2Mt_1$	87e	1.90f	188d	724c
$T2Mt_2$	204bc	4.90c	406b	986c
$T2Mt_3$	140d	2.60e	294c	1454b

Means with the same letter are no significantly difference at 5% level. M is the inoculated plants by *Meloidogyne javanica*. Control is the nontreated plants with nematode or fungi. In first time factor (TMt1), *Trichoderma* fungi and 2000 nematode larvaes was inoculated to the soil so that this soil had been mixed with peat and bran wheat ratio 1% and tomato three-week-seedlings were transmitted to the pots. In second time factor (TMt2), the soil was inoculated with fungi and nematode in time of transmission of three-week- seedlings. In third time factor (TMt3), *Trichoderma* fungi were inoculated to the soil and seedlings were transmitted and 2000 larvaes were inoculated to the soil after 12 days

Disease indices: The treatment of $T1Mt_1$ and $T2Mt_1$ decreased the gall number significantly ($p<0.05$). The effect of these treatments compared to nematode-infected nontreated plants, followed by 64 and 70%, respectively ($p<0.05$). The treatment of $T2Mt_3$ had the most effective after these treatments. The effect of other treatments was in range of 21 to 70%. The treatment of $T1Mt_2$ had the least effective compared to other treatments (Table 3). The effect of all treatments was significant compared to nematode-infected nontreated plants ($p<0.05$).

The treatment of $T2Mt_1$ had the most effective in decrease of gall diameter. The effect of other treatments was in range of 19 to 72% (Table 3). The effect of all treatments was significant compared to nematode-infected nontreated plants ($p<0.05$). The treatment of $T1Mt_2$ had the least effective in decrease of gall diameter compared to other treatments.

The T2Mt1 and T1Mt1 treatments had the most effective in the nematode penetration. These treatments decreased the nematode penetration, followed by 61 and 68%, respectively (Table 3). The effect of other treatments was in range of 23 to 68% (Table 3). The effect of all treatments was significant compared to nematode-infected nontreated plants ($p<0.05$). The treatment of $T1Mt_2$ had the least effective in decrease of nematode penetration compared to other treatments.

In treatments of $T2Mt_1$ and $T1Mt_1$, nematode population in soil was less than nematode-infected non treated plants ($p<0.05$). The effect of treatments in reduction of nematode population in soil was in range of 28 to 69% (Table 3). The effect of all treatments was significant compared to nematode-infected nontreated plants ($p<0.05$). The treatment of $T2Mt_1$ and $T1Mt_3$ had the most effective and the least effective in decrease of the soil nematode population compared to other treatments, respectively.

Trichoderma population was in range of 6.2 to 7.8 (Log CFU g^{-1}) in the soil of all pots (Table 3). In fungi treatments, T2 had the most population of fungi and in nematode-fungi treatments; $T2Mt_3$ had the most population of fungi in soil. T2 treatment had the most population of fungi and $T1Mt_2$ treatment had the least population of fungi in soil compared to other treatments respectively.

The treatment of $T2Mt_2$ and $T1Mt_2$ had the most population of fungi in rhizosphere compared to other treatments, respectively. The fungi population in rhizosphere was ranged 1.4-6.7 (Table 3).

In *Trichoderma* isolates, T2 isolate had the endophytice population more than T1 isolate. The endophytice population was in range of 1.2 to 4.8. The treatment of $T2Mt_1$ had the most endophyte population of fungi and $T2Mt_2$ treatment had the least endophytice population of fungi compared to other treatments, respectively (Table 3).

DISCUSSION

This study was aimed at elucidating the parasitic capabilities of *Trichoderma* isolates on the *M. javanica* and important of inoculated time of *Trichoderma* in their biocontrol activities against the nematode. Parasitism is probably an important mode of action and one of the initial steps of this process is attachment. The mention medium (PDB) in *in vitro* enabled fungal attachment and enhanced parasitic capabilities of the isolates compared to sterile water which could also utilize mention medium as a nutrient source. The mention medium has also been found to trigger proteolytic and chitinolytic enzyme production by the fungus (Sharon *et al*., 2007). This combination of enzymes is required to disrupt the egg shell (Tikhonov *et al*., 2002; Khan *et al*., 2004), although chitinolytic capacity is probably the most important activity on the egg-shells (Morton *et al*., 2004).

T1 isolate exhibited lower parasitic capabilities that may be related to insufficient chitinolytic activity of this isolate, while T2 isolate presented the major efficiency for parasitism of eggs (*in vitro*), probably it is because of its high chitinolytic activities. Production of chitin in this fungus has been studied and its involvement in fungal parasitism has been shown by Morton *et al*. (2004). T2 isolate exhibited lower parasitic capabilities that may be related to insufficient proteolytic activity of this isolate, while T1 isolate presented the major efficiency for parasitism of J2s (*in vitro*), that probably it is because of its high proteolytic activities (Sharon *et al*., 2007). Production of proteinase Prb1 in this fungus has been studied and its involvement in fungal parasitism has been shown (Flores *et al*., 1997).

The time of inoculation is important in biocontrol of nematode by *Trichoderma* sp. In this study, T2Mt1 was the best treatment to biocontrol of nematode. In this treatment, nematode was exposed with *Trichoderma* sp. isolates for 18 days then transplanted three-week-seedling. The concentration of the acidic component (s) in the soil is very low in first time of inoculation of *Trichoderma* and a certain period of time may was needed for its accumulation before its effect on J2 becomes significant. Thus in those experiments in which J2 was exposed with *Trichoderma* for 18 days prior planting, was observed maximum nematicidal efficacy; but when J2 were exposed to the fungus during planting. In treatments where *Trichoderma* had been assigned to the root-ball, was not recorded nematicidal activity. It is presumed, therefore, that nematodes were exposed with *Trichoderma* preparations in the soil over a 10-day period undergo paralysis, which delays their reaching the young roots at the initial stage of the seedling's exposure to the J2. For all studied species, the use of wheat bran increased the production of spore. However, the enhancement was extremely high for *T. harzianum* and *T. viride* nutritional supplementation needed production of high spores' (Cavalcante *et al*., 2008).

Application of the preplanted fungus to nematode-infested soil might enable the production of fungal metabolites with anti-nematode activity such as those found in the soil extracts, which could immobilize J2 and reduce nematode penetration. Moreover, via soil application, the fungus might directly parasitize the nematodes. When naturally infested soil was used, a longer preplanting incubation period was required to achieve significant control compared to J2-infested soil. This might be due to immediate exposure of J2 to the fungus and its metabolites in J2-infested soil, whereas in naturally infested soil, where gradual hatching of the eggs takes place, longer exposure to the *Trichoderma* preparation is required. The J2 cuticle and proteins is composed (Blaxster *et al*., 1998); therefore, we assumed that it is possible improvement of proteolytic activity of the antagonist has ultimated to increase biocontrol ability.

It is suggested that the main antinematode activity caused by *T. harzianum* takes place in the soil and not within the roots. An induced-resistance cascade can probably be excluded, because application of the *Trichoderma preparation* to the root system alone did not result in sufficient biological control, nor did it prevent nematode penetration or development inside the roots. This work supports the hypothesis that improvement of proteolytic activity of *Trichoderma* strains is important to the nematode biocontrol process in the both suggested mechanisms: effect of produced metabolites by the fungus in the soil and directed parasitism by the antagonist (Sharon *et al*., 2001).

In this study, top wet weight in $T2Mt_1$ and $T2Mt_3$ treatments had not significant difference (in 0.05 levels) but its difference was significant in disease indexies (in 0.05 levels). Top wet weight had significant increase in pre-colonization plants by *Trichoderma* sp. but they had not significant reduction in disease indexies (in 0.05 levels) also, Sharon *et al*. (2001) resulted that biocontrol of pre-colonization plants was not satisfactory but top wet weight had significant increase in these plants (in 0.05 levels). Treatment of the soil by *Trichoderma isolates* at the time of transplanting caused to the small reduction in nematode galling. However, total gall was reduced significantly when treatment was done one week before transplanting-allowing to extend exposure of the nematode against the antagonist. Our results are in agreement with those of Al-Fattah and Richard (2007).

In this research, larva mortality in percent and parasitism on nematode's egg was major by *Trichoderma* isolates that it may be because of production of proteolytic or chitinolytic enzyme by the fungus. The gm has also been found to trigger production of proteolytic and chitinolytic enzyme by the fungus (Sharon *et al*., 2007). This combination of enzymes requires to disrupt the eggshell (Tikhonov *et al*., 2002; Khan *et al*., 2004), although chitinolytic capacity is probably the most important activity on the eggshells (Morton *et al*., 2004) While T1 isolate played the major efficiency to parasite of J2s, probably because of its high proteolytic activities. However, secondary metabolites in fungi also contain compounds which are toxic to plant parasitic nematodes. *Trichoderma* may be effective as an egg parasitism; and a pre-plant treatment when eggs are present, not J2, may give better results (Al-Fattah and Richard, 2007). Additional studies are needed to clarify the interaction of *Meloidogyne* spp. with *T. harzianum* as a biocontrol agent in terms of the physiological roles of enzyme activities in response to attraction of nematode and fungal colonization (Abd-Elgawad and Kabeil, 2010).

In this research, the nematode population was very high in the soil of T1Mt3 and T2Mt3 treatments. The nematode penetration in root was less in the treatment of *Trichoderma* fungi and nematode simultaneity.

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