Research Article

Elicitation of defense responses in tomato against *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *Lycopersici* wilt complex

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Abstract: In this study, *Trichoderma harzianum* BI was evaluated for its capability to reduce the incidence and severity of the root-knot nematode, *Meloidogyne javanica,* and Fusarium wilt, *F. oxysporum* f. sp. *lycopersici,* as causal agents of a complex disease of tomato in the laboratory and greenhouse conditions. Initial *in vitro* studies revealed that the parasitism of *M. javanica* eggs by *T. harzianum* BI was up to 49.6%. In dual culture tests, maximum growth inhibition of *F. oxysporum* f. sp. *lycopersici* by *T. harzianum* BI (55%) was observed on the fifth day in laboratory. In greenhouse studies, the efficiency of treatments was appraised by using nematode-related factors such as diameter of galls, number of galls per plant, number of egg masses per plant and also incidence of Fusarium wilt. The antagonistic fungus was further tested for its ability to induce production of defense related enzymes in tomato. The activity of phenylalanine ammonia lyase (PAL) was increased significantly in the seedlings treated with the antagonistic fungus in comparison with control and its maximum amount was reached on the fourth day after inoculation with *T. harzianum* BI. Thus, the present study shows that in addition to direct antagonism, induction of defense-related enzymes, by *T. harzianum* BI that are involved in PAL pathway contributed to enhanced resistance against invasion of *M. javanica* and *F. oxysporum* f. sp. *lycopersici* in tomato.

Keywords: *Meloidogyne javanica*, *Fusarium oxysporum* f.sp. *lycopersici*, *Trichoderma harzianum* BI, Phenylalanine ammonia lyase (PAL)

Introduction

Plant-parasitic nematodes, especially the sedentary endoparasitic forms such as *Meloidogyne* species, are one of the important pests of tomato and greenhouse-grown crops, causing severe economic damages (Siddiqui and shaukat, 2003; Bird *et al.,* 2008; Nasr Esfahani *et al.,* 2012).

Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synder and Hans, is one of the major limiting factors in tomato-growing regions of Iran (Amini, 2009; Morid *et al.,* 2012). The symptoms of disease include yellowing of the lower leaves, wilting of leaves and defoliation, marginal necrosis of the remaining leaves, severe brown discoloration of vascular tissues along the stem and finally death of the plant (Agrios, 2005).

Studies in the past decades, have shown that interactions between *Meloidogyne* spp. and several special form of *Fusarium,* cause more damage and higher wilt severity than the sum damages of each agent alone (Hussey and Mc Guire, 1987; Starr *et al.,* 1989; France and Abawi, 1994).

Several researchers have demonstrated control of the root-knot nematode *Meloidogyne*s pp. and *Fusarim oxysporum* disease complex using various biocontrol organisms such as *Trichoderma* spp. (Sharma and Trivedi, 2002; Haseeb *et al.,* 2005; Nagesh *et al.,* 2006; Srivastava *et al.,* 2010). *Trichoderma harzianum* is able to parasitize nematode eggs and juveniles and penetrate nematode egg mass matrix (Sharon *et al.,* 2001). This fungus also is involved in induction of plant defence system against disease agents and induction of resistance in plants (Hibar *et al.,* 2007; Verma *et al.,* 2007). Ojha and Chatterjee (2012) investigated the effect of salicylic acid and *Trichoderma harzianum* on the induction of defense enzymes in tomato plants infected with *Fusarium oxysporum* f. sp. *lycopersici.* They indicated that total phenolic content, peroxidase and polyphenol oxidase activityincreased significantly in infected plants.

Srivastava *et al.* (2010) evaluated a large number of *Trichoderma* spp. and *Pseudomonas* isolates for their efficacy to control *Fusarium* wilt of tomato. They observed that *T. harzianum* significantly reduced the incidence of *Fusarium* wilt and increased seed germination. Isolates of *Trichoderma* also could reduce the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli.*, in vitro and reduced disease severity of pathogen in the greenhouse (Otadoh *et al.* 2011). Sharon *et al.* (2007) and Golzary *et al.* (2011) examined parasitism of *Trichoderma* isolates on different life stages of *M. javanica* in the laboratory and also in pot experiments on tomato. Their results indicated that egg masses, eggs and second-stage juveniles (J2) were parasitized by different *Trichoderma* isolates and that *Trichoderma* spp.

significantly reduced gall diameter, gall number and nematode population in treated roots.

In the present study, greenhouse experiments were done to evaluate the ability of *Trichoderma harzianum* BI to: (1) suppress the root-knot nematode, *Meloidogyne javanica* infection in tomato seedling; (2) decrease the *Fusarium* wilt caused by *F. oxysporum* f. sp. *lycopersici*; (3) control of *M. javanica* -*F. oxysporum* disease complex on *Solanum lycopersicum* var. Early urbana Y. and laboratory experiments were conducted to (4) monitor the induction of some resistance-related enzymes such as phenylalanine ammonia lyase (PAL) in tested plants.

Materials and Methods

The Pathogens
Soil and root samples were collected from the rhizoshere of tomato (*Solanum lycopersicum* Mill.) plants, from root- knot nematode infested fields of Pakdasht. Egg masses on galls were picked off and then allowed to hatch. Second-stage juveniles were inoculated to tomato seedlings. Following nematode detection based on Jepson (1987), single egg mass was used to establish the *M. javanica* culture on tomato plants.

Fusarium oxysporum f. sp. *Lycopersici* was obtained from Department of Plant Pathology, Faculty of Agriculture, Tehran University and cultured on potato dextrose agar (PDA) containing streptomycin and chloramphenicol, 150 mg per liter of each. The Petri dishes were then placed in an incubator at 25°C. Spores from 14-day-old cultures were collected gently from the surface of each culture plate with sterile distilled water. Then, spore concentration in the suspension was adjusted to final concentration of 10^6spores/ml with the aid of a Neubauer's chamber haemocytometer.

Biocontrol agent
Trichoderma harzianum BI was obtained from Department of Plant Pathology, Faculty of Agriculture, Tehran University and cultured on

PDA containing streptomycin and chloramphenicol, 150 mg per liter of each. The Petri dishes were then incubated at 25°C for six days, the spores were then collected from the plates with sterile distilled water and adjusted to final concentration of 10^6 spores/ml using a Neubauer's chamber haemocytometer.

Plant material
Experiments were executed on tomato (*Solanum lycopersicum* var. Early Urbana Y.) grown in a controlled-environment cabinet at ambient temperature of 25±3°C.

Laboratory experiments

Effect of *T. harzianum* BI on *M. javanica* eggs
Approximately, 30-40 eggs were transferred to the center of a glass slide bearing plugs of 10-days old culture of *T. harzianum* BI on each end. The glass slides were set on moistened sand contained in petri plates. Each treatment was replicated 4 times. Eggs placed on center of glass slide without the fungus served as control. Five days after incubation at 28°C in a dark room, eggs from *T. harzianum* BI treated and control slides, were separately examined under microscope. The experiment was replicated two times and data were taken on parasitism, mortality and destruction of eggs by *Trichoderma*.

Effect of *T. harzianum* BI on growth of *F. oxysporum* f. sp. *lycopersici*
In dual culture assays, 5 mm disc of 8–10 days old culture of pathogen was placed on one side of a petri plate and 48h later a disc of 6–7 days old culture of antagonist was placed on the opposite side of petri plate. Control petri plate was without culture of antagonist. Petri plates were then sealed with Parafilm and incubated at 27 ± 2 °C in dark for 5 days. Radial growth of pathogen was recorded and percentage of growth inhibition was calculated (Dennis and Webster, 1971). The experiment was replicated two times. The growth inhibition was calculated using formula I = (C-T)/C ×100, where I = percent growth inhibition, C = radial growth of pathogen in absence of antagonist, and T = radial growth of pathogen in presence of antagonist.

Greenhouse experiments
Tomato seeds were sown in 10 cm diameter pots containing sterilized mixture of field soil, leaf compost, and sand at the ratio of 2:1:1, and seedlings were then grown for 60 days in the greenhouse.
Seedlings at four-leaf stage received 20 ml of a suspension of *T. harzianum* BI containing 10^6 spores/ml and 20 ml of a suspension of *F. oxysporum* f. sp. *Lycopersici* (Sahebani and Hadavi, 2008). The nematode suspension (2000 J2 of *M. javanica* in water) was injected 2 cm deep into the rhizosphere using 3 holes around the stem base which were made by a plastic rod (Sahebani and Hadavi, 2008).

Pot inoculation of the seedlings with biocontrol agent and the 2 pathogens was applied with an interval of 4 days. Treatments shownin the parentheses were applied simultaneously and control pots were treated with distilled water only. The experiment consisted of 11 treatments: 1.- T +N, 2. -N+T, 3.-T + (F + N), 4.-(F+N)+T, 5.- T+F, 6.-F+T, 7.- N+F, 8.-only*T. Harzianum* T, 9.-only *F. oxysporum* f. sp. *Lycopersici* F, 10.-Only *M. javanica* N, and 11.-Uninoculated negative control. All of the pots were kept in the greenhouse at 25 °C ±3 with 16 h of supplemental artificial light per day.

The experiment was terminated 60 days after applications of the fungi and the nematode. The number of galls, egg masses, diameter of galls per plant (Mirehki *et al.,*2013) and symptoms of *Fusarium* wilt (number of yellowing and dropping of leaves) were then recorded (Mohamed and Haggag, 2006). The experiment was repeated for a second time. Statistical analysis was done with the SAS software package (ver. 9.1). ANOVA analysis was performed with critical difference at 5% level of significance.

Enzymatic activity determination
Enzymatic activity of phenylalanine ammonia lyase (PAL) was determined in treatments number 1, 3, 5, 7, 8, 9, 10 and 11. Root of seedling was inoculated with 10^6 spores/ml of antagonistic fungus at six-leaf stage by soil drenching. Each treatment was replicated 4 times. Four days after inoculation, plant roots were inoculated with 2000 nematode J2/plant and suspension of 10^6 spores of *F. oxysporum* f. sp. *lycopersici*. Root samplings were done daily for 7 days. Fresh tomato roots were washed and dried with filter paper, homogenized in mortar with liquid nitrogen. The homogenate was rinsed with the same volume of 0.1 M sodium phosphate buffer (PH 6.0) at 4 °C, and filtered through a 0.2 mm nylon filter into a centrifuge tube. The tissue extracts were centrifuged at 13,000 rpm for 20 min at 4 °C. The supernatant was used for enzymatic activity assay (Reuveni, 1995).

Enzymatic activity of (PAL) was assayed according to Chen *et al.* (2000) method. Specific enzyme activity was expressed based on the amount of trans-cinnamic acid in reaction mixture, at 290 nm per ml of plant exudates. The experiment was performed in four replicates.

Results

Laboratory experiments
T. harzianum BI caused significant infection of *M. javanica* eggs by direct parasitism (Fig. 1). The percentage of unhatched eggs treated with *T. harzianum* was 49.6%, in contrast to 12.3% in control. The percentage of growth inhibition in *F. oxysporum* f. sp. *Lycopersici* caused by *T. harzianum* BI ranged from 25% to 55.3% from day 1 to 5, although growth of *Fusarium* was inhibited steadily, there was no significant difference in radial growth of mycelium between day one to day three and also between days four and five. *Trichoderma* increasingly inhibited growth of *F. oxysporum* f. sp. *lycopersici*

and its antagonistic capability increased with time (Fig. 2).

Greenhouse experiments
The soil treated with *T. harzianum* BI either before or after inoculation with nematode and *Fusarium*, separately or in combination (F+N) significantly reduced disease severity indices such as diameter of gall, number of galls and number of egg massess per plant as well as wilt incidence in comparison to controls (F+N). Significant reduction in gall formation was obtained with *T. harzianum* BI, when applied before and after nematode and *Fusarium* inoculation, separately or simultaneously (Fig. 3). *T. harzianum* BI reduced diameter of galls when applied before nematode infection and in simultaneous inoculations of nematode and *Fusarium* (Fig. 4). *T. harzianum* BI significantly reduced number of egg masses in all treatments (Fig. 5), whereas, these parameters increased in simultaneous inoculation of nematode and *Fusarium* (F+N). Application of *T. harzianum* before and after inoculation with *Fusarium* and also in simultaneous inoculations with a combination of nematode and *Fusarium* reduced the incidence of wilt in pot trials (Fig. 6). In other words, in all the experiments, *T. harzianum* BI gave good levels of control.

Enzymatic activity determination
Inoculation of tomato roots with *T. harzianum* BI, significantly increased PAL enzyme activity as compared to un-inoculated control. Maximum level of enzyme activity was achieved four days after inoculation by *T. Harzianum* BI, and then it slowly decreased. In plants inoculated with nematode alone, PAL activity was reduced significantly within one to four days after inoculation, and then increased gradually. In plants that were inoculated only with *F. oxysporum* f. sp. *lycopersici*, PAL activity showed no significant change during the sampling days (Fig. 7).
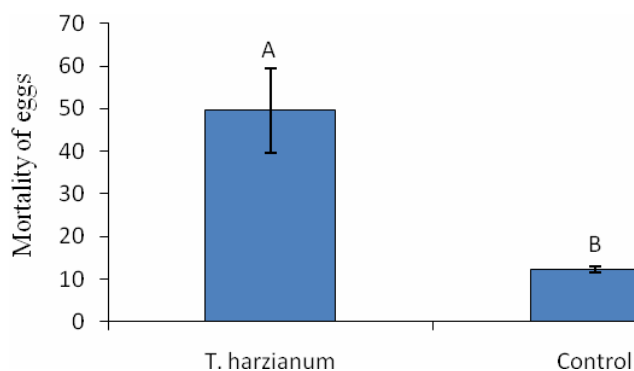
Figure 1 Effect of *T. harzianum* BI on *M. javanica* eggs mortality as compared to control. Means with different letters are significantly different (Duncan's test, P ≤0.05; n = 4).
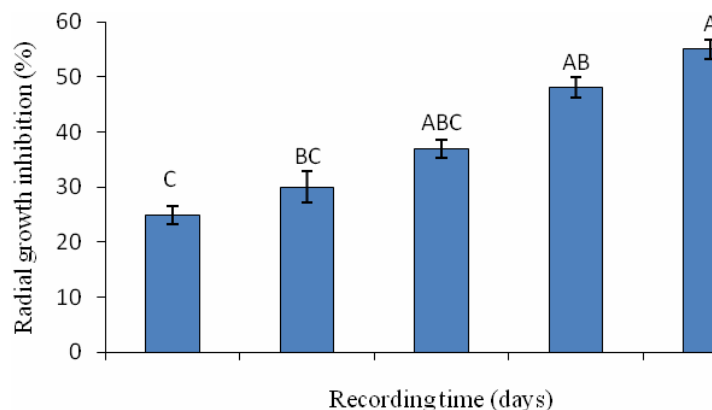

Figure 2 Radial growth inhibition of *F. oxysporum* f. sp. *Lycopersici* by *T. harzianum* BI in course of time, day 1 to 5. Means with different letters on the column are significantly different (Duncan's test, P ≤0.05; n = 4).
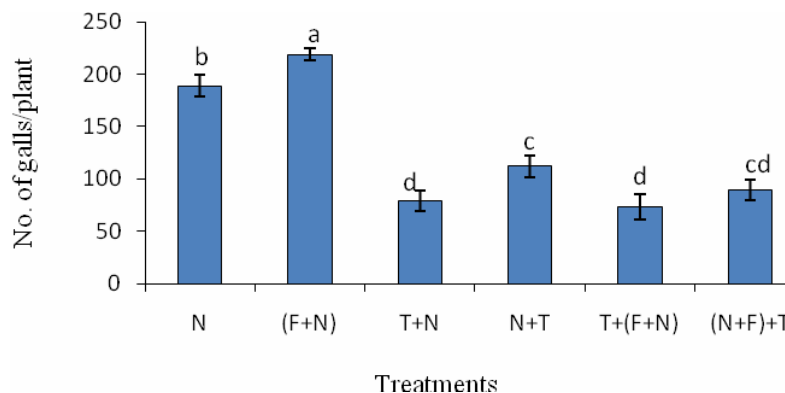

Figure 3 Effect of *T. harzianum* BI (T) on number of galls formed by *M. javanica* alone (N) and in combination with *F. oxysporum* f. sp. *lycopersici* (F + N). Treatments within parentheses are applied simultaneously. Means with different letters on the columns are significantly different (Duncan's test, P ≤ 0.05; n = 4).
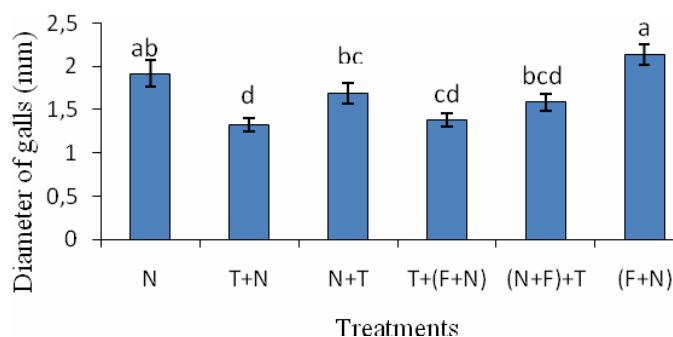
Figure 4 Effect of *T. harzianum* BI (T) on diameter of galls formed by *M. javanica*alone (N) and in combination with *F. oxysporum* f. sp. *lycopersici* (F + N). Treatments within parentheses are applied simultaneously. Means with different letters on the columns are significantly different (Duncan's test, P ≤ 0.05; n = 4).
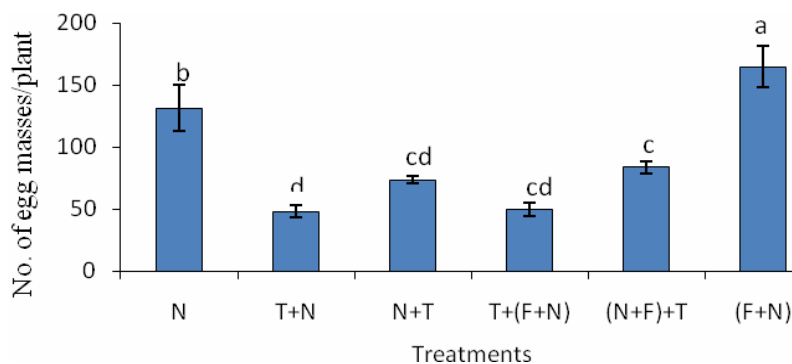

Figure 5 Effect of *T. harzianum* BI (T) on mean number of egg masses formed by *M. javanica*alone (N) and in combination with *F. oxysporum* f. sp. *Lycopersici* (F+N). Treatments within parentheses are applied simultaneously. Means with different letters on the columns are significantly different from each other (Duncan's test, P ≤ 0.05; n = 4).
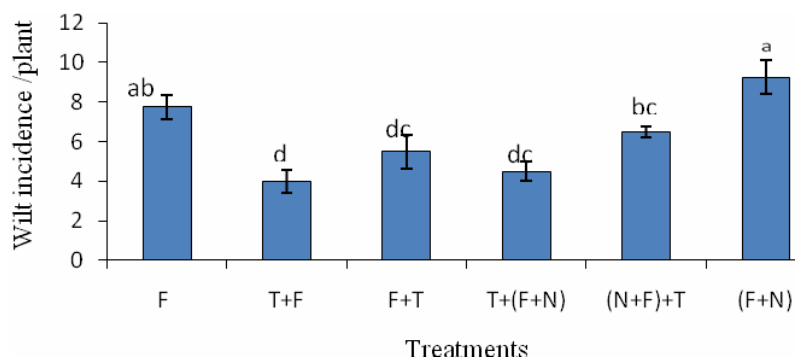

Figure 6 Effect of *T. harzianum* BI (T) on wilt incidence of leaves in tomato plants caused by *F. oxysporum* f. sp. *lycopersici* alone (F) and in combination with *M. javanica* (F+N). Treatments within parentheses are applied simultaneously. Means with different letters on the columns are significantly different (Duncan's test, P ≤ 0.05; n = 4).
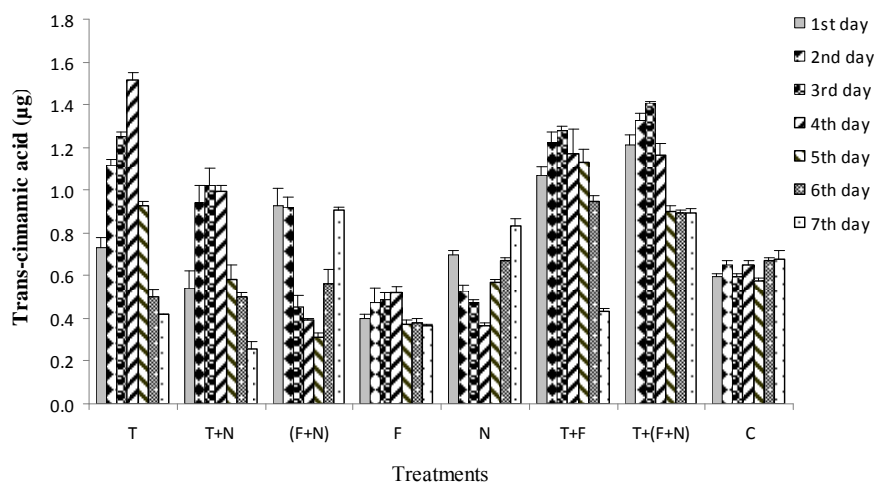
Figure 7 Phenylalanine ammonia lyase specific activity in tomato roots (var. Early Urbana Y) as estimated by releasing of trans-cinnamic acid from phenylalanine, on days 1 to 7 after inoculation with *T. harzianum* BI (T), *M. javanica* (N), *F. oxysporum* f. sp. *Lycopersici* (F) and different combinations of T, N and F. Each value represents the mean of four replicates. The bars correspond to standard error.

Discussion

The results of our laboratory and greenhouse experiments indicate that *T. Harzianum* BI has significant biocontrol potential against tested pathogens, the root-knot nematode *M. javanica* and *F. oxysporum* f. sp. *lycopersici*. The most important aspect of this study was the efficacy of *T. harzianum* BI against the disease complex of the root-knot nematode and wilt fungus. Our experiments showed that this biocontrol agent has a good performance against both pathogens either singly or in their simultaneous presence as disease complex agents.

T. harzianum BI significantly reduced the hatching of *M. javanica* eggs. These results are in agreement with results of Naserinasab *et al.* (2010). In their experiment, *T. harazianum* significantly reduced the egg hatching of *M. javanica* so that the percentage of unhached eggs was 84% as compared to 21% in control. *Trichoderma* spp. have been reported to produce chitinases (Chet and Baker, 1981) which might inhibit the eggs from hatching. Sharon *et al.* (2001) showed direct parasitism of *T. harzianum* (T-203) on *M. javanica* under *in vitro* condition.

In this study, the maximum percentage of growth inhibition of *F. oxysporum* f. sp. *Lycopersici* by *T. harzianum* was observed as 48.5% and 55.3% after four and five days, respectively. Similar results were obtained by Srivastava *et al.* (2010) who reported that maximum percentage of growth inhibition of *F. oxysporum* f. sp. *lycopersici* by *T. harzianum* isolates ranged from 30% to 48%.

Application of *T. harzianum* to the soil or nursery beds of tomato plants can significantly reduce population density of *Meloidogyne* and the severity of *Fusarium* wilt. Soil treatment with *T. harzianum* BI resulted in a reduction in the numbers of galls, egg masses and percentage of *Fusarium* wilt incidence. But application of *T. harzianum* to the soil, prior to the pathogenic agents, was more effective. This suggests that root colonization by *T. harzianum* can induce the production of defense-related enzymes and probably other defense compounds which lead to systemic resistance in plants. Our results indicate that *T. harzianum* BI has direct and indirect effects on nematode infection by reducing egg hatch and induction of resistance in plant. Sharon *et al.* (2001) reported that *T. harzianum* reduced galling of root-knot nematode *M. javanica* on tomato

plants. Results of this study also showed that *T. harzianum* BI significantly increased PAL activity in tested plant as compared to the control. Ziarati *et al.*, (2009) measured changes of total phenolics in tomato infected by nematode during different times and observed that there was induced systemic resistance in tomato plant by *Trichoderma* fungus against *M. javanica*. This suggests that *T. harzianum* BI can induce production of defense-related enzymes and probably other defense compounds leading to systemic resistance in plants. Root colonization by *T. harzianum* BI, has been reported to increase defense level related to plant enzymes including various peroxidase, chitinase, β-1,3-glucanases, lipoxigenase and phenylalanine ammonia lyase (Sahebani and Hadavi, 2008; Naserinasab *et al.*, 2010; Howell *et al.*, 2000; Yedidia *et al.*, 1999; Evans *et al.*, 2003). The biocontrol level was significantly improved, when *T. harzianum* BI was applied before nematode and *Fusarium*. The results of our study suggest that *T. harzianum* BI can be used as a very effective biocontrol agent to protect tomato plants from *F. oxysporum* f. sp. *lycopersici* and *M. javanica*.

References

Agrios, G. N. 2005. Plant Pathology, 5th edition, Academic Press, USA.

Amini, J. 2009. Physiological race of *Fusarium oxysporum* f. sp. *lycopersici*in Kurdistan province of Iran and reaction of some tomato cultivars to race 1 of pathogen. Journal of Plant Pathology, 8: 68-73.

Bird, D. M. cK., Opperman, C. H., and Williamson, V. M. 2008. Plant Infection by Root-Knot Nematode. Plant Cell Monographs: 1-5.

Chen, C., Belanger, R. R., Benhamou, N. and Paulitz, T. C. 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Phytium aphanidermatum*. Physiological and Molecular Plant Pathology, 56: 13-23.

Chet, I. and Baker, R. 1981. Induction of suppressiveness to *Rhizoctonia solani*in soil. Phytopathology. 70: 994-998.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma.*

III. Hyphal interactions. Transactions of the British Mycological Society, 57: 363-369.

Evans, H. C., Holmes, K. A., Thomas, K. A. 2003. Mycobiota of an indigenous *Theobroma* species (Sterculiaceae) in Ecuador: assessing its potential for biological control of cocoa diseases. Mycology Progress, 2: 149-160.

France, R. A. and Abawi, G. S. 1994. Interaction between *Meloidogyne incognita* and *Fusarium oxysporurm* f, sp. *Phaseoli* on Selected Bean Genotypes. Journal of Nematology, 26 (4): 467-474.

Golzary, H., Panjehkeh, N., Ahmadzadeh, M., Salari, M. and Sedaghati-khoravi, E. 2011. Elucidating the parasitic capabilities of *Trichoderma* against *Meloidogyne javanica* on tomato. Insight Plant Disease, 1: 12-19.

Haseeb, A., Sharma, A., Shukla, P. K**.** 2005. Studies on the management of root-knot nematode, *Meloidogyne incognita*-wilt fungus, *Fusarium oxysporum* disease complex of green gram, *Vignar adiata* cv ML-1108. Journal of Zhejiang University Science, 6 (8):736-742.

Hibar, K., Daami-Remadi, M. and El Mahjoub, M. 2007. Induction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *radicis-lycopersici* by *Trichoderma* spp. Tunisian Journal of Plant Protection, 2: 47-58.

Howell, C. R., Hanson, L. E., Stipanovic, R. D., Puckhaber, L. S. 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathology, 90: 248-252.

Hussey, R. S. and McGuire, J. M. 1987. Interactions with other organisms. In: Brown, R. H. and Kerry, B. R. (Eds.), Principles and Practice of Nematode Control in Crops. Academic Press, Marrickville, Australia, pp. 294-320.

Jepson, S. B. 1987. Identification of root knot nematodes (*Meloidogyne* species). CAB International. United Kingdom, 256 p.

Mirehki, K., Abdollahi, M., and Talaee, F. 2013. Effect of *Trichoderma virens* and*Glomus mosseae* in control of *Meloidogyne javanica* in tomato. Biological Control of Pests and Plant Disease, 2: 9-16.

Mohamed, H. A. L. A. and Haggag. W. M. 2006. Biocontrol potential of salinity tolerant mutants of *T. harzianum* against *Fusarium oxysporum*. Brazilian Journal of Microbiology, 37: 181-191.

Morid, B., Hajmansoor, S, and Kakvan, N. 2012. Screening of resistance genes to *fusarium* root rot and *fusarium* wilt diseases in tomato (*Lycopersicom esculentum*) cultivars using RAPD and CAPs markers. European Journal of Experimental Biology, 2: 931-939.

Nagesh, M., Hussaini, S. S., Ramanujam, B. and Chidanandaswamy, B. S. 2006. Management of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopercisi* wilt complex using antagonist fungi in tomato. Nematologia Mediterranea, 34: 63-68.

Naserinasab, F., Sahebani, N., and Etebarian, H.R. 2010. Biological control of *Meloidogyne javanica* by *Trichoderma harzianum* BI and salicylic acid on Tomato. African Journal of Food Science, 5 (3): 276-280.

Nasr Esfahani, M., Ahmadi, A. R. and Shirazi, k. 2012. Susceptibility Assessments of Tomato Genotypes to Root-Knot Nematodes, *Meloidogyne javanica*. Journal of Ornamental and Horticultural Plants, 2 (2): 113-121.

Ojha, S. and Chatterjee, N. C. 2012. Induction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *lycopersici* mediated through salicylic acid and *Trichoderma harzianum*. Journal of Plant Protection Research, 52: 220-225.

Reuveni, R. 1995. Biochemical marker of disease resistance. In: Singh, R. P. and Singh, U. S. (Eds.),. Molecular Methods in Plant Pathology, pp. 99-114.

Sahebani, N. and Hadavi, N. 2008. Biological control of the root-knot nematode *Meloidogyne javanica*by *Trichoderma harzianum*. Soil Biology Biochemistry, 40: 2016-2020.

Sharma, N. and Trivedi, P. C. 2002. Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against *Meloidogyne incognita* and *Fusarium oxysporum*. Asian Journal of Experimental Sciences, 16: 21-28.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O. and Spiegel, Y. 2001. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology, 91: 687-693.

Sharon, E., Chet, I., Viterbo, A., Bar-Eyal, M., Nagan, H., Samuels, G. J. and Spiegel, Y. 2007. Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. European Journal of Plant Pathology, 118: 247-258.

Siddiqui, I. A. and S. S. Shaukat. 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHAO in tomato: Importance of bacterial secondary metabolite, 2,4-D diacetyloholorglucinol. Soil Biology and Biochemistry, 35: 1615-1623.

Srivastava, R., Khalid, A., Singh, U. S. and Sharma, A. K. 2010. Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biological Control, 53: 24-31.

Starr, J. L., Jeger, M. J., Martyn, R. D. and Schilling, K. 1989. Effects of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *Vasinfectum* on plant mortality and yield of cotton. Phytopathology, 79: 640-646.

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y. and Valero, J. R. 2007. Antagonistic fungi, *Trichoderma* sp.: Panopy of biological control. Biochemical Engineering Journal, 37: 1-20.

Yedidia, I., Benhamou, N. and Chet, I. 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Applied Environmental Microbiology, 65: 1061-1070.

Ziarati, H. M., Roustaee, A., Sahebani, N., Etebarian, H. and Aminian, H. 2009. Study of biological control of root-knot nematode, *Meloidogyne javanica* (Trube) Chitwood, in tomato by *Trichoderma harzianum* Rifai in greenhouse and quantitative changes of phenolic compounds in plant. Seed and Plant Production Journal, 25: 259-272.

القاء پاسخ دفاعی در گوجه‌فرنگی توسط *Trichoderma harzianum* BI علیه بیماری مختلط نماتد ریشه گرهی *Meloidogyne javanica* و قارچ پژمرگی آوندی فوزاریومی .f *Fusarium oxysporum* sp. *lycopersici*

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چکیده: در این تحقیق، توانایی *T. harzianum* BI در کنترل بیماری مختلط نماتد ریشه گرهی (*M. javanica*) و قارچ عامل پژمردگی آوندی (*F. oxysporum* f. sp. *lycopersici*) در آزمایشگاه و گلخانه در گیاه گوجه‌فرنگی مورد ارزیابی قرار گرفت. مطالعات آزمایشگاهی نشان داد قارچ آنتاگونیست سبب پارازیتیسم مستقیم تخم نماتد تا ۴۹/۶ درصد شد و در آزمایش‌های کشت دوگانه حداکثر درصد بازداری از رشد میسیلیوم قارچ پژمردگی آوندی به‌میزان ۵۵٪ در روز پنجم مشاهده شد. به‌علاوه در آزمایش‌های گلخانه‌ای شاخص‌های بیماریزایی؛ از قبیل تعداد گال‌ها، تعداد توده تخم، قطر گال و شاخص پژمردگی برگ‌ها ارزیابی شد. هم‌چنین توانایی این قارچ در القاء آنزیم‌های دفاعی در گوجه‌فرنگی بررسی شد. فعالیت آنزیم فنیل آلانین آمونیالیاز (PAL) به‌طور چشم‌گیری در گیاهچه‌های تیمار شده با قارچ آنتاگونیست در مقایسه با شاهد افزایش یافت و حداکثر میزان فعالیت این آنزیم در روز چهارم بعد از مایه‌زنی با آنتاگونیست مشاهده شد. بنابراین *T. harzianum* BI علاوه بر ضدیت مستقیم با عوامل بیمارگر، در القاء آنزیم‌های دفاعی گیاه؛ از جمله مسیر PAL، در افزایش مقاومت گیاه علیه نماتد مولد گره ریشه و قارچ پژمردگی آوندی در گوجه‌فرنگی نقش دارد.

واژگان کلیدی: قارچ آنتاگونیست، *Trichoderma harzianum* BI، نماتد مولد گره ریشه *Meloidogyne javanica*، قارچ عامل پژمردگی آوندی گوجه‌فرنگی *Fusarium oxysporum* f.sp. *lycopersici*، فنیل آلانین آمونیالیاز (PAL)