African Journal of Biotechnology Vol. 10(31), pp. 5850-5855, 29 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1600
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake

Rasool Azarmi[1], Behzad Hajieghrari[2]* and Abolfazl Giglou[1]

[1]Department of Plant Production, Moghan Junior College of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran.
[2]Department of Bioinformatics, Institute of Biophysics and Biochemistry (IBB), University of Tehran, Tehran, Iran.

Accepted 4 April, 2011

Trichoderma **species are commonly used as biological control agents against phytopathogenic fungi and some isolates are able to improve plant growth. In this study, the effects of three** ***Trichoderma*** **isolates including** ***Trichoderma harzianum*** **isolate T969,** ***T. harzianum*** **isolate T447 and** ***Trichoderma*** **sp. isolate T in tomato seedling vigor and their nutrient uptake via two inoculants introduction methods (inoculating seed with** ***Trichoderma*** **spore suspension and inoculating nursery soil with** ***Trichoderma*** **fortified wheat) were examined. Seed germination rate was not affected by** ***Trichoderma*** **application, but shoot height, shoot diameter, shoot fresh and dry weight and root fresh and dry weight in tomato seedlings were interestingly ($p \leq 0.05$) increased when sown in** ***Trichoderma*** **sp. T and** ***T. harzianum*** **T969 fortified soil and when compared to the control. The soil amended by** ***Trichoderma*** **sp. T and** ***T. harzianum*** **T969 had marked increase in leaf number and leaf area ($p \leq 0.05$). Chlorophyll content increased in seedling grown in** ***Trichoderma*** **sp. T amended soil as well as in** ***Trichoderma*** **sp. T and** ***T. harzianum*** **T969 coated seed. A dramatic increase ($p \leq 0.05$) in the concentrations of Ca^{2+}, Mg^{2+}, P and K^+ were recorded in the seedling shoot and root among** ***T. harzianum*** **T447 soil amended treatment when compared to the control, except for Na^+ level in soil amendment with** ***T. harzianum*** **T969 and seed-coating with strain** ***Trichoderma*** **sp. T, which significantly reduced the Na^+ concentration.**

Key words: Growth response, nutrient uptake, tomato seedling, *Trichoderma harzianum*.

INTRODUCTION

Fungal species belonging to the genus *Trichoderma* are common filamentous imperfect saprophytic fungi in soil and rhizosphere ecosystem that have been known not only for their potential to control several commercial phytopathogens that caused soil-borne (Koch, 1999; Spiegel and Chet, 1998; Barker and Paulitz, 1996; Harman and Hadar, 1983), air-borne (Elad, 2000) and post harvest (Freeman et al., 2004) diseases in a wide range of crops by different mechanisms (Howell, 2003), but also for their ability to promote plant growth (Hoyos-Carvajal et al., 2009; Shanmugaiah et al., 2009; Harman et al., 2004; Ousley et al., 1994; Barker, 1989; Barker, 1988) and improve nutrient uptake (Chet, 2001; Yedidia et al., 2001), as well as improve plant defense level against biotic and/or abiotic stress (Mastouri et al., 2010; Hoitink et al., 2006; Honson and Howell, 2004; Yedidia et al., 1999). Plant growth enhancement by *Trichoderma* isolates is as a result of different mechanisms such as exudation of plant growth regulators and/or their similarity with the fungi (Hoitink et al., 2006; Vinale et al., 2008a; Culter et al., 1989; Windham et al., 1986), solubilization of phosphates, micronutrient and minerals such as Fe, Mn and Mg that have important role in plant growth (Altomare et al., 1999), secretion of exogenous enzymes, sidrophores (Jalal et al., 1987) and vitamins (Inbar et al., 1994; Kleifeld and Chet, 1992), as well as indirectly with the control of the major and minor root infesting pathogens (Harman et al., 2004) in rhizosphere. The variety of some of these mechanisms indicate multiple modes of action (Harman, 2006; Harman et al., 2004) that lead to increase in nutrient availability and uptake, resulting in the stronger nutrient uptake by plant, and thereby developing the root system.

*Corresponding author. E-mail: bhajieghrari@uma.ac.ir. Tel: +989143186861. Fax: +984527463417

Beside the other necessary factors in its growth, it makes better support for shoot growth and development. The effect of *Trichoderma* isolates on plant growth and development is important, especially in nursery, because improvement of plant vigor to overcome biotic and/or abiotic stresses results in the production of stronger plants and increase in plant productivity and yields.

There are relatively few strains of *Trichoderma* that have the ability to stimulate plant growth response (Lo and Lin, 2002). The most beneficial *Trichoderma* strains that are able to colonize the root and inhabit the rhizosphere are known to have the "rhizosphere competence" (Harman et al., 2004; Ahmad and Baker, 1987). Therefore, screening of *Trichoderma* isolates is beneficial in enhancing plant growth and development, which is highly desirable in order to reduce or eliminate the use of synthetic chemical fertilizers from the point of the view of sustainable agricultural system because application of man-made fertilizer is not economical in the long run for environmental pollution, due to the fact that harmful residues and their highly application cost are left in the soil.

Recently, some researchers have however, reported the effect of *Trichoderma* isolates directly on the plant growth parameters in some commercial crops (Shanmugaiah et al., 2009; Bal and Altintas, 2008; Babeendran et al., 2000; Zheng and Shetty, 2000; Phuwiwat and Soytong, 1999; Lynch et al., 1991). Particularly, Chacon et al. (2007) showed that *Trichoderma harzianum* is able to promote tomato plant growth by colonizing the roots, increasing the foliar area and secondary roots, as well as changing the root system architecture under sterile condition (Bjorkman et al. 1999). In contrast, Bal and Altinas (2006) demonstrated that application of *T. harzianum* did not increase yield in tomato. *De facto*, the effect of *Trichoderma* on plant growth improvement is not the result of *Trichoderma* isolate and plant species, but also the complex interaction of many factors may have an influence on the *Trichoderma*-plant interaction such as environmental parameters, soil microorganisms and soil-plant interaction (Harman et al., 2004).

The purpose of this study was to examine three *Trichoderma* isolates including *T. harzianum* isolate T969, *T. harzianum* isolate T447 and *Trichoderma sp* isolate T in tomato seedling vigor and their nutrient uptake via two inoculants introduction methods.

MATERIALS AND METHODS

Material preparation

The experiment was carried out at the Biology laboratory and greenhouse of the Department of Plant Production, Moghan Junior College of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran during summer 2009.

Two of the *Trichoderma* isolates that were selected for this study were obtained from the collection of *Trichoderma* spp., in the Plant Pest and Disease Institute, Tehran, Iran and they included: *T. harzianum* isolate T969 and *T. harzianum* isolate T447. A non-determined species from *Trichoderma* genus as *Trichoderma* sp. T was isolated from Moghan wheat field soil. The isolates were grown and maintained on potato dextrose agar (PDA, BDH Ltd, UK 39 g/l) medium and were stored at 4°C for further use. Also, tomato (*Lycopersicum esculentum*) cultivar 'super β' was selected for this study.

Inoculation methods

In this experiment, for evaluating of the effect of *Trichoderma* isolates on tomato seedling vigor and growth improvement, two inoculation methods were tested. In one method, the tomato seeds were coated with *Trichoderma* spore suspension. For the preparation of the spore suspension, 5 mm diameter mycelia disc of 7 days-old culture obtained from the margin of each *Trichoderma* isolate was centrally placed on the surface of 100 ml PDA in a 250 ml conical flask and was incubated at 25 ± 1°C for 7 days. After the incubation period, 30 ml of double distilled water (ddH_2O) was added to each conical flask and was shaken on a rotary shaker at 80 rpm for 30 min. The concentration of *Trichoderma* spores in ddH_2O was counted using haemocytometer and was adjusted to 10^6-10^7 spores per ml. Five tomato surface disinfected seeds, soaked in 0.5% hypochlorite sodium (NaClO) for 5 min and then rinsed and washed thoroughly in sterile distilled water 3 times, were inoculated by immersion in 1 ml of the spore suspension for 30 min. Subsequently, they were sown in each pot, while the control seeds were immersed in 1 ml of the sterile distilled water.

In the second inoculation method, the *Trichoderma* isolates were cultured on sterilized wheat and the fortified wheat was added to the nursery soil. In order do to this, five discs of mycelia agar plugs obtained from the margin of each *Trichoderma* isolates (one week old growing colonies) were removed with No. 3 cork borer (5 mm diameter) and were added to 1 kg of sterilized wheat grain in 1 L conical flasks (autoclaved twice at 121°C for 30 min with 24 h interval after adding 10 ml distilled water) and then incubated at 25 ± 1°C for two weeks before it was mixed with soil in a 1:5 ratio. However, the control conical flasks were inoculated with five discs of 5 mm diameter sterile PDA medium.

Physiological and biochemical measurements

Plant growth response parameter including total chlorophyll content, total leaf area, leaf chlorophyll fluorescence and stomata conductance were measured 45 days after planting in each plant via chlorophyll meter (Model: SPAD 502 Konika Minolta Sensing Inc, Japan), leaf area meter (Model: Li 3100, Area meter Licor Lincon Nebraska, USA), chlorophyll flourometer (SPDA 502 Konika Minolta Seasing Inc, Japan) and portable steady state porometer (Model: SC-1, Eijkel Kamp, Netherlands) instruments, respectively.

Also, plant height, stem diameter, root elongation, shoot and root fresh, dry weight, leaf number, as well as shoot and root Ca^{2+}, Mg^{2+}, K^+, Na^+ and P contents were measured after uprooting and washing the plants under running tap water to remove residual soil from the roots.

For evaluation of the minerals' content, the samples were dried at 80°C for 48 h and then placed overnight in a muffle furnace at 500°C to give a gray ash. After cooling, 10 ml of the 6 M HCl were added and then boiled on water bath until dryness was evaporated. Also, this stage was repeated with 2 ml high concentration of HCl again. Afterwards, 10 ml of double distilled water were added to the dry gray, heated to boiling and was filtered through a millipore filter in order to remove the residues.

Table 1. Effect of *Trichoderma* isolates and the process of application to tomato seed in seedling vigour.

Treatment	Seedling height (cm)	Crown diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Soil amended treatment						
Control	7.33 (±2.25)^{cd}	0.217 (±0.03)^{c}	2.367 (±0.96)^{bc}	1.18 (±0.05)^{b}	0.683 (±0.44)^{bc}	0.14 (±0.02)^{b}
Trichoderma sp.	17.17 (±3.51)^{a}	0.530 (±0.03)^{a}	19.43 (±1.96)^{a}	2.07 (±0.12)^{a}	2.873 (±1.12)^{a}	1.91 (±0.05)^{a}
T. harzianum T969	17.75 (±1.56)^{a}	0.517 (±0.03)^{a}	19.98 (±2.81)^{a}	2.1 (±0.07)^{a}	3.240 (±0.91)^{a}	2.02 (±0.03)^{a}
T. harzianum T447	4.33 (±0.76)^{d}	0.140 (±0.04)^{d}	0.587 (±.34)^{c}	0.145 (±0.11)^{b}	0.237 (±0.18)^{c}	0.14 (±0.05)^{b}
Seed inoculated treatment						
Control	7.66 (±1.89)^{cd}	0.198 (±0.04)^{c}	2.423 (±0.92)^{bc}	1.09 (±0.08)^{b}	0.653 (±0.54)^{bc}	0.13 (±0.03)^{b}
Trichoderma sp.	11.17 (±1.04)^{b}	0.287 (±0.07)^{b}	4.240 (±1.73)^{b}	1.307 (±0.05)^{b}	1.287 (±0.15)^{bc}	0.75 (±0.04)^{b}
T. harzianum T969	9.67 (±1.61)^{bc}	0.273 (±0.03)^{bc}	3.647 (±0.56)^{b}	1.28 (±0.03)^{b}	1.033 (±0.32)^{bc}	0.64 (±0.01)^{b}
T. harzianum T447	9.17 (±0.58)^{bc}	0.253 (±0.03)^{bc}	3.813 (±0.13)^{b}	1.267 (±0.01)^{b}	1.577 (±0.65)^{b}	0.95 (±0)^{b}

Values with the same letter within the column were not significantly different (P≤ 0.05) according to Duncan's test results. Results are means of four replicates for each treatment. The value in parentheses is the standard deviation of the mean.

The phosphorous content of samples was measured by vanado-molibodate indication method. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) ion contents of the samples were determined by complexometery with EDTA (Rowell, 1996), while sodium (Na^{+}) and potassium (K^{+}) ion concentrations were estimated by flame photometer (PEP 7 and PEP 7/C, Jen way).

Microbial activity assay

Production of CO_2 was also measured as a soil microbial activity indicator in all the treatments. In order to evaluate the soil CO_2 production, soil samples were incubated in an hermetic flask for 7 days at 25°C and the produced CO_2 was trapped in excess of 0.5 M NaOH, after which the trapped CO_2 was titrated to phenolphthalein with HCl in the presence of $BaCl_2$. Thus, the difference between the released CO_2 in the different treatments was calculated (Rowell, 1996).

Statistical analysis

The experimental design used in this study was a completely randomized design (CRD) in four replicates for each treatment. The means were analyzed by analysis of variance (ANOVA) and Duncan's test at 5% significant level with SAS software [SAS (1985) Institute Inc. Cary, NC, USA].

RESULTS

The effects of different *Trichoderma* isolates and their application method on seed germination, seedling growth promotion and vigor were observed as early as 45 days after seed potting. The results presented here showed that there were no significant ($p \leq 0.05$) differences between the tomato seed germination and the seedling emergence rate in all the tested treatments when compared with the non-inoculated pots.

As shown in Table 1, sharp ($p \leq 0.05$) increases in shoot height, shoot diameter, shoot fresh and dry weight and root fresh and dry weight in tomato seedlings were observed when sown in *Trichoderma* sp. isolate T and *T. harzianum* T969 fortified soil when compared with the control (Table 1). Seedling height, crown diameter, shoot fresh and dry weight, and root fresh and dry weight also increased in seed inoculated treatments by *Trichoderma* isolates, but the increases were not significantly ($p \geq 0.05$) different except for *Trichoderma s*p isolate T in the shoot height and diameter compared to that of the untreated pot (Table1).

According to the evidence shown in Table 2, the soil amended by *Trichoderma* sp and *T. harzianum* T969 led to an increase in leaf number and area markedly ($p \leq 0.05$). Chlorophyll content, although not significantly ($p \geq 0.05$) increased, was higher in the leaves of tomato seedling sowed in the amended soil and seed coating treatment by *T. harzianum* T969 (Table 2). Leaf chlorophyll fluorescent was significantly ($p \leq 0.05$) increased in the *Trichoderma* sp. fortified treatment, whereas stomata conductivity was not significantly ($p \geq 0.05$) affected (Table 2).

The mineral content in the shoot and root of the treated tomato seedling is shown in Table 3. Based on the obtained data, a dramatic increase ($p \leq 0.05$) in the concentration of Ca^{2+}, Mg^{2+}, P and K^{+} but not Na^{+} was recorded in the seedling shoot in *T. harzianum* T447 amended soil treatment when compared to the control. Also, no significant ($p \geq 0.05$) differences were found among the Ca^{2+}, Mg^{2+}, P, Na^{+} and K^{+} content of the shoot in *Trichoderma* amended soil and *Trichoderma* seed inoculated seedlings in relation to their controls, except for Na^{+} content where a significant ($p \leq 0.05$) decrease in the seedlings grown in *T. harzianum* T969 inoculated soil and *Trichoderma* sp. isolate T inoculated seed treatments was observed when compared to their controls.

Root mineral content analysis revealed prominent ($p \leq$

Table 2. Effect of *Trichoderma* isolates and the process of application to tomato seed in seedling physiological parameters.

Treatment	Leaf number	Leaf area (cm^2)	Chlorophyll content	Photochemistry	Stomata conductivity (m $Molm^{-2}s^{-1}$)	Soil respiration
Soil amended treatment						
Control	3 (± 0.87)^{c}	30.54 (±19.01)^{b}	32.37 (±0.4)^{ab}	4.55 (± 0.48)^{bc}	149.6 (±12.61)^{a}	1.694 (±1.37)^{bc}
Trichoderma sp.	5.33 (±1.26)^{a}	303.5 (±32.29)^{a}	31.5 (±3.41)^{b}	9.41 (± 1.19)^{a}	82.53 (±48.60)^{a}	3.922 (±0.61)^{a}
T. harzianum T969	5.67 (±0.26)^{a}	333.8 (±98.1)^{a}	34.57(±0.25)^{a}	6.15 (±2.39)^{b}	91.07(±42.94)^{a}	3.72 (±0.43)^{a}
T. harzianum T447	1.83 (±0.58)^{d}	27.53 (±1.09)^{b}	25.6 (±0.62)^{c}	2.59 (±1.31)^{c}	60.51(±23.15)^{a}	0.684(±0.49)^{c}
Seed inoculated treatment						
Control	3.11(±0.77)^{c}	31.34 (±18.98)^{b}	33.57 (±0.35)^{ab}	4.35 (± 0.53)^{bc}	144.7 (±11.23)^{a}	1.546 (±1.54)^{bc}
Trichoderma sp.	4.16(±0.29)^{b}	58.00 (±26.73)^{b}	32.47 (±1.63)^{ab}	3.06 (± 0.83)^{c}	129.2 (±32.41)^{a}	3.095 (±0.13)^{ab}
T. harzianum T969	3.5(±0.00)^{bc}	54.85 (±11.11)^{b}	33.27 (±0.92)^{ab}	2.31 (±0.40)^{c}	109.6 (±38.43)^{a}	2.945 (±1.02)^{ab}
T. harzianum T447	3.5(±0.00)^{bc}	37.10 (±7.34)^{b}	31.73 (±0.76)^{b}	2.50 (± 0.37)^{c}	113.2 (±45.07)^{a}	2.976 (±1.02)^{ab}

Values with the same letter within the column were not significantly different (P≤ 0.05) according to Duncan's test results. Results are means of four replicates for each treatment. The value in parentheses is the standard deviation of the mean.

Table 3. Effect of *Trichoderma* isolates and the process of application to tomato seed in seedling shoot and root elements content.

Treatment	Shoot					Root			
	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Na (g/kg)	K (g/kg)	Ca (g/kg)	Mg (g/kg)	P (g/kg)	K (g/kg)
Soil amended treatment									
Control	34.41 (±1.99)^{bc}	22.41 (±5.39)^{b}	3.24 (±1.01)^{b}	1.323 (±0.03)^{ab}	1.232 (±0.21)^{bcd}	10.2 (±2)^{c}	3.11 (±0.1)^{d}	0.365 (±0.38)^{b}	0.107 (±0.01)^{d}
Trichoderma sp.	28.74 (±0.79)^{bc}	14.34 (±1.19)^{b}	3.683 (±0.52)^{b}	1.032 (±0.46)^{abc}	6.33 (±1.34)^{b}	19.37 (±3)^{b}	5.81 (±0.2)^{c}	0.387(±0.45)^{b}	0.367 (±0.03)^{c}
T. harzianum T969	23.13 (±5.29)^{c}	16.57 (±3.48)^{b}	5.439 (±1.79)^{ab}	0.496 (±0.36)^{c}	5.38 (±0.62)^{bc}	10.407 (±1.9)^{c}	5.083 (±0.55)^{c}	0.54 (±0.3)^{b}	0.302 (±0.01)^{c}
T. harzianum T447	223.4 (±3.91)^{a}	70.44 (±26.86)^{a}	7.674 (±0.33)^{a}	1.762 (±0.78)^{a}	10.68 (±2.93)^{a}	57.033 (±1.91)^{a}	11.443 (±1.53)^{b}	8.889 (±1.14)^{a}	1.143 (±0.08)^{a}
Seed inoculated treatment									
Control	34.56 (±2.3)^{bc}	22.53 (±4.23)^{b}	3.18 (±1.2)^{b}	1.331 (±0.14)^{ab}	1.253 (±0.23)^{bcd}	9.81 (±2.3)^{c}	3.15 (±0.21)^{d}	0.345 (±0.34)^{b}	0.100 (±0.03)^{d}
Trichoderma sp.	26.05 (±5.05)^{bc}	11.99 (±5.91)^{b}	4.597 (±2.93)^{b}	0.431 (±0.36)^{c}	1.121 (±0.05)^{d}	21.45(±3)^{b}	2.97 (±0.79)^{d}	0.587 (±0.2)^{b}	0.123 (±0.09)^{d}
T. harzianum T969	35 (±6.65)^{bc}	18.2 9 (±3.69)^{b}	4.63 (±2.23)^{b}	1.131 (±0.25)^{abc}	3.17 (±0.1)^{bcd}	23.33 (±3.2)^{b}	17.4 (±1)^{a}	0.41 (±0.2)^{b}	0.386 (±0.02)^{c}

Values with the same letter within the column were not significantly different (P ≤ 0.05) according to Duncan's test results. Results are means of four replicates for each treatment. The value in parentheses is the standard deviation of the mean.

0.05) increases in the concentration of Ca^{2+}, Mg^{2+}, P and K^{+} content in *T. harzianum* T447 amended soil treatment in comparison to the control. Meanwhile, Ca^{2+} concentration in *Trichoderma* treated seedlings was markedly (p≤0.05) increased when compared with their control, except for *T. harzianum* T969 enriched soil treatment where the Ca^{2+} content was similar to its control. Also, Mg^{2+} content in the root of *T. harzianum* T969 and *T. harzianum* T447 seed inoculated treatments were significantly (p ≤ 0.05) higher than that in the non-inoculated seedling.

The same result was also observed for K^+ concentration in the root of treated seedlings. However, there was no significant ($p \geq 0.05$) difference in P content except for *T. harzianum* T969 supplemented soil treatment. The K^+ content was significantly ($p \leq 0.05$) improved in all the treatments except in *Trichoderma* sp. isolate T inoculated seed treatment that was statistically similar with that of its controls.

DISCUSSION

Trichoderma spp. employs several mechanisms in influencing seed germination and seedling vigor (Zheng and Shetty, 2000; Clear and Valic, 2005). Seed germination rate, rapidity of root elongation and development during seed germination, plant height, root fresh and dry weight, and shoot fresh and dry weight of seedling are the most important indicators of seedling vigor.

In this study, seed germination rate was not affected by *Trichoderma* application ($p \geq 0.05$). Seedling height, crown diameter, shoot fresh and dry weight, and root fresh and dry weight, as well as leaf number and total area of leaves were increased significantly by applying *T. harzianum* T969 and *Trichoderma* sp isolate T via *Trichoderma*-fortified wheat grain. However, application of *T. harzianum* T447 inoculated wheat grains in the soil mainly reduced the aforementioned factors. This result indicated that the effects of *Trichoderma* on seedling growth and vigor consistently depend on *Trichoderma* species/isolate applied. This finding is consistent with the results of other authors (Hajieghrari, 2010; Ousley et al., 1994; Barker, 1988).

On the contrary, in the *Trichoderma* seed inoculation treatments, no significant effect was observed in seedling height, shoot fresh and dry weight, root fresh and dry weight, leaf number and total area. These results indicate that the method of *Trichoderma* introduction is also effective in the success of *Trichoderma* isolate in seedling growth improvement.

This clearly indicated that the increased growth response of plants, caused by *Trichoderma*, depended mainly on the ability of *Trichoderma* to survive and develop in the rhizosphere (Harman, 2006; Harman et al., 2004). Root colonization by *Trichoderma* could be a result of not only the root exudates such as carbohydrates and amino acid, but also by many factors that affected *Trichoderma*-plant interaction. In this regard, some *Trichoderma* isolates may interact better with the plant in the same conditions. The result as observed in this study revealed that root development of seedling in *T. harzianum* T969 and *Trichoderma* sp T occurred in *Trichoderma*-fortified wheat grain treatments.

The work of other researchers show that the rhizosphere competent isolate produces diffusible metabolites in the rhizosphere which actively influence the growth of *Trichoderma*–colonized plant due to their action as plant growth regulators (auxin and/or auxin-like compound) (Vinale et al., 2008a, b). These compounds have an optimum activity at low concentrations, while they have an inhibitory effect at high doses (Vinale et al., 2008a, b). This condition may justify the observed inhibitory effect of the *T. harzianum* T447 inoculated treatment. Nonetheless, these materials may lead to the development of the root system and an exploration of a large volume of soil. Development of the root system with production of some organic acids in the rhizosphere such as gluconic, citric and/or fumaric acids by *Trichoderma* which decrease soil pH, lead to increased solubility of the insoluble compound and an availability of micronutrient, as well as an increase in plant nutrient uptake. Improvement of plant nutrient uptake and its transport from root to aerial parts, together with the produced plant stimulators, might result in higher photosynthetic rates required for producing enough energy used to derive the enhanced growth response. This hypothesis is supported by the obtained result of *Trichoderma* sp and *T. harzianum* T969 treatment especially in the soil amended treatment because of the high density of the *Trichoderma* population.

The results presented here confirm that the concentration of Ca^{2+}, Mg^{2+}, P, Na^+ and K^+ increased in the shoot and root following the application of *T. harzianum* T447 in the soil. First, higher levels of the elements in the treated plants indicated that transport mechanisms of these elements from root to shoot were also induced. However, growth in these treated seedlings was decreased when compared to the control. This effect could be as a result of the high production of stimulant factors by *T. harzianum* T447 that have an inhibitory effect at higher doses than the optimal concentration. This hypothesis is also supported by other works (Vinale et al., 2008a, b). In this regard, there are some reports demonstrating pathogenicity of some *Trichoderma* isolates among some crops (Hajieghrari, 2010; Menzies, 1993; Mc-Fadden and Sutton, 1975; Sutton, 1972). Meanwhile, the production of some antibiotics by *T. harzianum* T447 might be the reason for the reduction of soil respiration in the *T. harzianum* T447 amended soil, indicating a reduction of soil microbial activities. Although the other *Trichoderma* treated soil respiration increased when compared to the controls, no significant increase was observed by them. Consequently, more detailed studies are still needed among the various isolates of *Trichoderma* species in order to provide a better understanding of the mechanisms of promoting or inhibiting plant growth responses.

ACKNOWLEDGMENT

The author wishes to thank the University of Mohaghegh Ardabili, Ardabil, Iran for financially supporting this

research through a scientific research grant offered.

REFERENCES

Ahmad JS, Baker R (1987). Rhizosphere competence of *Trichoderma harzianum*. Phytopathol. 77(2): 182-189.

Altomare C, Novell WA, Bjorkman T, Harman GE (1999). Solubilization of phosphate and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol. 65: 2926-2933.

Babeendean N, Moot DJ, Jones EE, Stewart A (2000). Inconsistent growth promotion of Cabbage and Lettuce from *Trichoderma* isolates. New Zealand Plant Protection, 53: 143-146.

Bal U, Altintas S (2008). Effects of *Trichoderma harzianum* on lettuce in protected cultivation. J. Cent. Eur. Agric. 9(1): 63-70.

Barker R (1988). *Trichoderma spp*. as plant stimulants. CRC Crit. Rev. Biotechnol. 7: 97-106.

Barker R (1989). Improved *Trichoderma spp*. for promoting crop productivity. Trends Biotechnol. 7: 34-38.

Barker R, Paulitz TC (1996). Theoretical basis for microbial interactions leading to biological control of soil borne plant pathogens. In: Hall R (Ed). Principals and practice of managing soil borne plant pathogens. Ann. Phythopathol. Soc. St. Paul, Mn. pp. 50-79.

Chacon MR, Rodriguez-Galan O, Beritez T, Sousa S, Rey M, Llobell A, Delgado-Jarana J (2007). Microscopic and transcriptome analyses of early colonization of tomato roots by *Trichoderma harzianum*. Int. Microbiol., 10: 19-27.

Chet I (2001). Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil, 235: 235-242.

Clear F, Valic N (2005). Effects of *Trichoderma spp* and *Glicladium roseum* culture filtrates on seed germination of vegetables and maize. J. Plant Dis. Prot. 112(4): 343-350.

Cutler HG, Himmellsbach DS, Arrendale RF, Cole PD, Cox RH (1989). Koninginin A: a novel plant growth regulator from *Trichoderma koningii*. Agric. Biol. Chem. 53: 2605-2611.

Elad Y (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot. 19: 709-714.

Freeman S, Minz D, Kolesnik I, Barbul O, Zreibil A, Maymon M, Nitzani Y, Kirshner B, Rav-David D, Bilu A, Shafir S, Elad Y (2004). *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea*, and survival in strawberry. Eur. J. Plant Pathol. 110: 361-370.

Hajieghrari B (2010). Effects of some Iranian *Trichoderma* isolates on maize seed germination and seedling vigor. Afr. J. Biotechnol. 9(28): 4342-4347.

Harman GE (2006). Overview of mechanisms and uses of *Trichoderma spp.* Phytopathol. 96(2):190-194.

Harman GE, Hadar Y (1983). Biological control of *Pythium* species. Seed Sci. Technol. 11: 893-906.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004). *Trichoderma* species- opportunistic, avirulent plant symbionts. Nature Reviewer, 2: 43-56.

Hoitink HAJ, Madden LV, Dorrance AE (2006). Systemic resistance induced by *Trichoderma spp*; Interactions between the host, the pathogens, the biocontrol agent and soil organic matter quality. Phytopathology, 96(2):186-189.

Honson LE, Howell CR (2004). Elicitors of plant defencse responses from biocntrol strains of *Trichoderma virens*. Phytopathology, 94(2): 171-176.

Howell CR (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant disease; the history and evolution of current concepts. Plant Dis. 87: 4-10.

Hoyos-Carvajal L, Ordua S, Bissett J (2009). Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. Biological Control, 51: 409-416.

Inbar J, Abramsky M, Cohen D, Chet I (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings growth under commercial conditions. Euro. J. Plant Pathol., 100: 337- 346.

Jalal MAF, Love SK, Vander-Helm D (1987). Siderophore mediated iron III uptake in *Gliocladium virens* (*Trichoderma virens*). 2. Role of ferric mono- and dihydroxamates as iron transport agent. J. Inorganic Biochem. 29: 259-267.

Kleifeld O, Chet I (1992). *Trichoderma harzianum* interaction with plants and effects on growth response. Plant Soil, 144: 267-272.

Koch E (1999). Evaluation of commercial products for microbial control of soil borne plant disease. Crop Prod. 18: 119-125.

Lo CT, Lin CY (2002). Screening strains of *Trichoderma spp* for plant growth enhancement in Taiwan. Plant Pathol. Bull. 11: 215-220.

Lynch JM, Wilson KL, Ousley MA, Wipps JM (1991). Response of lettuce to *Trichoderma* treatment. Lett. Appl. Microbiol. 12: 59-61.

Mastouri F, Bjorkman K, Harman GH (2010). Seed treated with Trichoderma harzianum alleviates biotic, abiotic, and physiological streeses in germinating seed and seedlings. Phytopathology, 100(11): 1213-1221.

Mc-Fadden AG, Sutton JC (1975). Relationship of populations of *Trichoderma spp* in soil to disease in Maize. Can. J. Plant Sci. 55: 579-586.

Menzies JG (1993). A strain of *Trichoderma viride* pathogenic to germinating seedlings of cucumber, paper and tomato. Plant Pathol. 42: 784-791.

Ousley MA, Lynch JM, Whipps JM (1994). Potential of *Trichoderma spp*. as consistent plant growth stimulators. Biol. Fertil. Soils, 17: 85-90.

Phuwiwat W, Soytong K (1999). Growth and yield response of Chinese radish to application of *Trichoderma harzianum*. Thammasat Int. J. Sci. Technol. 4(1): 68-71.

Robeendran N, Moot DJ, Jones EE, Stewart A (2000). Inconsistent growth promotion of cabbage and lettuce from *Trichoderma* isolates. New Zealand Plant Prot. 53: 143-146.

Rowell DL (1996). Soil science: methods and applications. Addison Wesley Longman Ltd. UK, p. 350.

Shanmugaiah V, Balasubramanian N, Gomathinayagam S, Monoharan PT, Rajendran A (2009). Effect of single application of *Trichoderma viride* and *Pseudomonas fluorences* on growth promotion in cotton plants. Afr. J. Agric. Res. 4(11): 1220-1225.

Spiegel Y, Chet I (1998). Evaluation of *Trichoderma spp*. as biocontrol agents soil borne fungi and plant parasitic nematodes in Israel. Integr. Pest Manage. Rev. 3: 169-175.

Statistical Analysis Software (SAS) (1985). Users Guide: Statistics version 5 Edition. SAS Institute Inc. Cary. NC. p. 956.

Sutton J (1972). *Trichoderma koningii* as a parasite of maize seedlings. Can. J. Plant Sci. 52: 1037-1042.

Vinale F, Sivasithamparam K, Ghisalberti E L, Marra R, Barbetti M J, Li H, Woo SL, Lorito M (2008a). A noel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiol. Mol. Plant Pathol. 72: 80-86.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, Lorito M (2008b). *Trichoderma*-plant-pathogen interactions. Soil Biol. Biochem. 40: 1-10.

Windham MT, Elad Y, Barker R (1986). A mechanism for increased plant growth Induced by *Trichoderma spp*. Phytopathology, 76(5): 518-521.

Yedidia I, Benhamou N, Chet I (1999). Induction of defense responses in cucumber (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 65: 1061-1070.

Yedidia I, Srivastva A K, Kapuluik Y, Chet I (2001). Effect of *Trichoderma harzianum* on microelement concentration and increased growth of cucumber plants. Plant Soil. 235: 235-242.

Zheng Z, Shetty K (2000). Enhancement of pea (*Pisum sativm*) seedling vigor and associated phenolic content by extraction of apple pomace fermented with *Trichoderma spp*. Proc. Bioch. 36: 79-84.