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Growth-promoting activity of indigenous Trichoderma isolates on wheat seed germination, seedling growth and yield

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Abstract

Apart from their biocontrol potential, *Trichoderma* rhizosphere-competent strains stimulate plants growth via several direct mechanisms that affect various growth parameters. In this study, effect of five indigenous isolates (*T. harzianum* T 969, *T. harzianum* T 447, *T. hamatum* T 614, *Trichoderma* sp. isolate T and *Gliocladium virens* G525.) on wheat (variety Moghan 3) seed germination, seedling vigor and plant growth was examined through seeds treatment by the conidia and culture filtrate of the isolates. In the field experiments various growth parameters including rootlet and shoot length, stem height and weight, root length and weight, total leaf area, head and tiller number and 1000 grain weight as well as the plant total chlorophyll content and stomata conductance response were evaluated. In overall, seed germination rate was increased. The highest seed germination rate (95.8%) was observed for *T. harzianum* T969 non-sterilized cultural filtrate treatment and after 96 hours. The isolate *G. virens* G525 spore treatment decreased the rate by 54.5%. Although all isolates colonized on rootlets but no significant effect on the seedlings growth was observed. Isolate *T. hamatum* T614 caused necrotic reaction on rootlets, revealing its parasitic rather than symbiotic activity. In the field experiments, the isolates had significant effect on parameters such as stomata conductance, 1000 grain weight, ear fresh and dry weights, ear length and stem and root dry weight but they showed no significant effect on other evaluated parameters. Some isolates improved the affected parameters and some had negative effects which are discussed.

Keywords: *Trichoderma*; wheat; seed germination; seedling vigor; plant growth.

Introduction

Agricultural soils around the world are under increasing pressure by input of huge amounts of chemical fertilizers to ensure crops high productivity and yield for growing human population. However, considering the many negative environmental and health consequences attributed to the use of inorganic fertilizers in agriculture and in order to achieve a sustainable and environmental-friendly agriculture, the use of such substances should be reduced and other eco-friendly and nature-based substances should be developed for improvements in the crops growth and yield (Gentili and Jumpponen, 2006, Hermosa et al., 2012, Bhardwaj et al., 2014). Biofertilizers are one of the environmental-friendly alternatives to the chemical fertilizers that are being marketed with the aim to increase the soils fertility and the crops productivity and yield without causing harmful environmental effects. These products also play important role in organic farming (Mihov and Tringovska, 2010, Hermosa et al., 2012). Biofertilizers are based on the microorganisms (fungi and rhizobacteria) that are able to colonize rhizosphere of the plants and promote plants growth and enhance their tolerance to the biotic and abiotic stresses via various mechanisms (Fuentes-Ramirez and Caballero-Mellado, 2005; Naganada et al., 2010). The fungus *Trichoderma* (Teleomorph: *Hypocrea*, an ascomycete), a ubiquitous fungus and common inhabitant of all soils and rhizospheres, and also common symbiont of the plants roots, has been an important component of biofertilizers (Vinale et al., 2014, Sharma et al., 2012). It is a biocontrol agent that beside antagonistic capabilities in suppression and control of soil-borne plant pathogenic fungi has also shown promising plant growth promoting potential (Harman et al., 2004, Druzhinina et al., 2011, Raats, 2012, Martínez-Medina et al., 2014). As an effective biocontrol agent, *Trichoderma* has been an important component of biofungicides with the aim to promote plants health avoiding the many negative effects of chemical fungicides on environment and human health (Lu et al., 2004, Topolovec-Pintarić et al., 2013, Maag et al., 2014). However, the results of investigations have highlighted the fungus abilities in enhancing plants growth independent from its antagonistic potential in suppression of plant pathogens (Contreras-Cornejo et al., 2009, Topolovec-Pintarić et al., 2013). Beneficial effects of *Trichoderma* as plant growth stimulator and promoter are attributed to several discovered mechanisms which include root colonization as endophyte symbiont, solubilization of the minerals and increasing availability of insoluble nutrients and therefore plant nutrient uptake, secretion of siderophores, plant growth regulatory materials (phytohormons), vitamins and enzymes (such as phosphate-solubilizing enzymes) (Anke et al., 1991, Benítez et al., 2004, Harman, 2006, Gravel et al., 2007, de Santiago et al., 2011, Li et al., 2015). The plant growth promoting activity of *Trichoderma* has been demonstrated in

a wide variety of crops including tobacco, tomato and radish (Windham et al., 1986), strawberries (Porras et al., 2007), lettuce (Bal and Altintas, 2008), onion (Altintas and Bal, 2008), cotton (Shanmugaiah et al., 2009), tomato (Gravel et al., 2007, Morsy et al., 2009), bean (Hoyos-Carvajal et al., 2009, Raats, 2012, Erper et al., 2013), soybean (John et al., 2010, Entesari et al., 2013), chickpea (Yadav et al., 2011), tulip (Mazahebi et al., 2011), apple (Raman, 2012), sunflower (Badar and Qureshi, 2012), wheat (Sharma et al., 2012), cabbage and red beet (Topolovec-Pintarić et al., 2013), rice (Doni et al., 2014a,b), gray mangroves (Saravanakumar et al., 2013) and potato (Hicks et al., 2014). Search for *Trichoderma* species, strains and isolates with higher growth-promoting activity is one of the fields of interest for scientists around the world. This is because the plant growth promoting potential varies amongst different *Trichoderma* species and strains, and also indigenous isolates are usually favored for subsequent use as biofertilizer (Martínez-Medina et al., 2014). These isolates can be formulated in combination with other rhizomicrobes and marketed for use as replacement to the chemical fertilizers.

Despite of intensive chemical fertilizers application and due to various abiotic stresses especially drought, the average yield of wheat crop, the main food crop in Iran, is low. Locally formulated biofertilizers also are not diversely available in the market for diverse number of crops grown countrywide, including wheat. Search for and screening of the indigenous microorganisms with higher growth-promoting potential to be used for manufacturing biofertilizers is essential step towards achieving satisfactory average yield and reduction of chemical fertilizers application. Thus, addressing this necessity and for the first time the plant growth promoting potential of some indigenous *Trichoderma* isolates on a local and most-cultivated wheat cultivar in Iran and their effect on various growth parameters such as seed germination, seedling growth and grain yield was studied.

Results

Effect of Trichoderma isolates on wheat seed germination and seedling vigor

Effect of sterilized and non-sterilized *Trichoderma* isolates cultural filtrate was examined on wheat seed germination, seedling height and rootlet length, crown diameter, shoot and rootlet fresh weight and leaf number. Sterilized and non-sterilized metabolites had marked effect ($P \leq 0.01$) on seed germination rate (89.22b for sterilized *Trichoderma* metabolite *vs.* 94.56a for non-sterilized *Trichoderma* metabolite; the main effect of metabolite sterilization). Non-sterilized filtrate significantly ($P \leq 0.01$) affected seed germination but had no effect on final seedling rootlet and shoot length ($P \geq 0.05$) two weeks after placing inoculated seeds on sterile blotting paper. In heat-sterilized cultural filtrate treatments no significant difference was observed among the treatments and the controls (non-inoculated and double distilled watered seeds) since all had no effect on seed germination and rootlet and shoot length ($P \geq 0.05$). The highest seed germination rate was observed for *T. harzianum* T969 non-sterilized cultural filtrate and 96 hours after treatment (Fig. 1). Seed germination rate in *T. harzianum* T447 non-sterilized cultural filtrate treatment and control was the same and other three *Trichoderma* treatments had no significant effect compared with the control and *T. harzianum T969* cultural filtrate treatment (Fig. 1). Direct inoculation of seeds by *Trichoderma* isolates spores had significant effect ($P \leq 0.01$) on seed germination but had no effect on seedling rootlet and shoot length and weight ($P \geq 0.05$). *T. harzianum* T969 spores inoculation significantly ($P \leq 0.01$) increased seed germination while inoculation of the seeds by *G. virens* G525 spores decreased and other three *Trichoderma* treatments had no effect on seed germination (Fig. 2). Microscopic observation of the roots of the seedlings grown in presence of *Trichoderma* isolates revealed complete attachment of the fungal hyphae to the root surface. Large mass of fungal hyphae grown on roots surface presented colonization of the rhizosphere by the isolates and intimate contact with the host root exodermises (Fig. 3, A). Transverse section of the root in *T. hamatum* T614 -treated seedlings showed that the fungus was able to penetrate the root epidermis and progress towards the cortical area mainly by intercellular growth facilitating fungal spread into the root tissue (Fig. 3, B and C). *T. hamatum* T614 penetrated the root system without causing extensive damage in initial stages (Fig. 3, D and E). In the early stages the cells might be sensitized to trigger the transient elaboration of the host defense reaction and respond faster to greater extent to potential pathogen attacks. In this sense, necrotic region was observed in the inner layer of rootlet emerged from *T. hamatum* T614 -inoculated seeds indicating its parasitic rather than symbiotic function (Fig. 4). *T. hamatum* T614 was re-isolated from the necrotic region.
The effect of *Trichoderma* isolates on culture media was also evaluated and it was observed that *Trichoderma* isolates significantly decreased the pH and affected EC and K+ and Na+ content of the media (Table 1).

Effect of Trichoderma isolates on wheat growth in field condition

No marked ($P \geq 0.05$) differences were observed between the two experimental fields in two separate locations. Results of both field experiments showed that treatment of wheat plants with *Trichoderma* isolates had statistically significant effect ($P \leq 0.05$) on stomata conductivity, 1000 grain weight, ear fresh and dry weights, ear length, and stem and root dry weight, but had no effect on other evaluated parameters. Stomatal conductance was significantly ($P \leq 0.05$) affected by *Trichoderma* isolates. It was significantly decreased ($P \leq 0.05$) in *T. harzianum* T447-treated wheat plants comparing with the control (Fig. 5). Significantly great values ($P \leq 0.05$) were obtained in grain weight for *Trichoderma*-treated wheat plants compared with the control. However, *G. virens* G525 treatment showed the greatest increase in grain weight (Fig. 6). Ear dry weight was significantly ($P \leq 0.05$) higher for *T. harzianum* T969 -treated wheat plants. Other *Trichoderma* isolates did not show significant effect compared with the control except for *T. hamatum* T614 that significantly ($P \leq 0.05$) decreased ear dry weight (Fig. 7). In contrast, *Trichoderma*-treated wheat plants showed significant decrease in ear fresh weight. Amongst the isolates, *T. hamatum* T614 had the greatest effect on ear fresh weight (Fig. 7).

It was also observed that the length of wheat ear was significantly ($P \leq 0.05$) shorter in *Trichoderma*-treated plants in comparison with the control. In particular, *T. hamatum* T614 had greater effect in reducing the ear length compared to the other isolates (Fig. 8). In addition, in *Trichoderma*-treated wheat plants stem and root dry weight was decreased. Significant decrease ($P \leq 0.05$) was observed in *G. virens* G525-treated plants (Fig. 9). However, no significant decrease in stem and root dry weight was seen in other *Trichoderma* isolates-treated wheat plants (Fig. 9). No

Table 1. Effect of *Trichoderma* isolates/species on culture medium.

Treatments	pH	EC (mho/cm)	Na^+ (ppm)	K^+(ppm)
Control (No inoculation)	6.64	2.7	5.272	5.009
Trichoderma harzianum T969	2.88	2.72	4.840	4.980
Trichoderma harzianum T447	3.4	2.37	5.542	5.330
Trichoderma sp. T	2.87	2.94	6.022	5.886
Gliocladium virens G525	4.2	2.76	2.864	5.477
Trichoderma hamatum T614	3.01	2.56	4.142	5.097

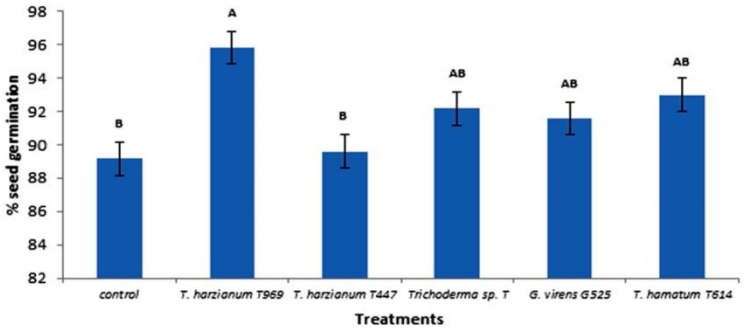

Fig 1. Effect of *Trichoderma* isolates non-sterilized cultural filtrate on wheat seed germination 96 hours after treatment (The main effect of *Trichoderma* isolates). Data are expressed as % of germinated seeds and values are average of 4 replications. Means with the same letter are not significantly different (P≥0.01) by Duncan's Multiple Range Test for variable.

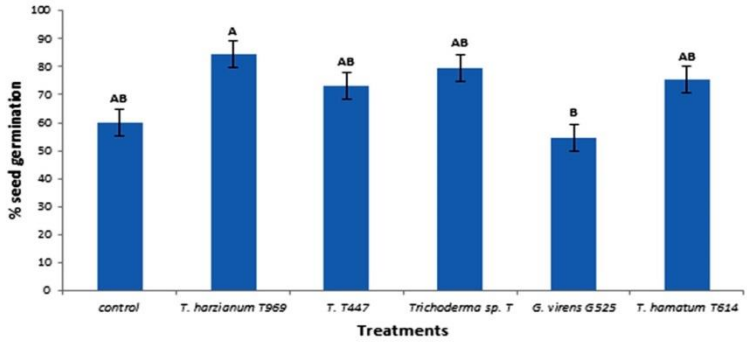

Fig 2. Effect of *Trichoderma* isolates spore inoculation on wheat seed germination (The main effect of *Trichoderma* isolates). Data are expressed as % of germinated seeds and values are average of 4 replications. Means with the same letter are not significantly different (P≥0.01) by Duncan's Multiple Range Test for variable.

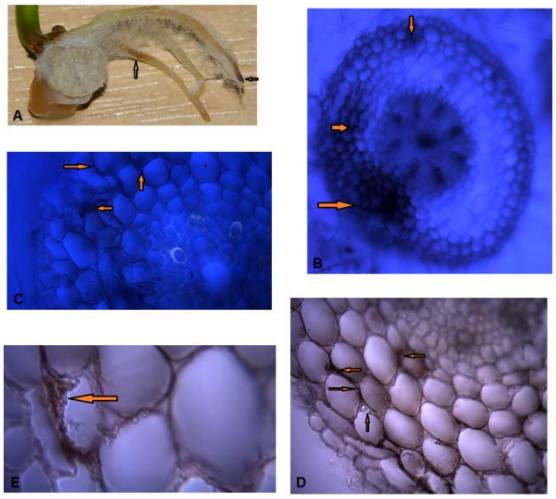

Fig 3. Colonization of the wheat rootlets by *Trichoderma hamatum* T.614, the isolate which showed parasitic activity and caused necrotic area in rootlet cortex; a large mass of fungal hyphae developed on the rootlets and seed surface as indicated by arrow (**A**). Its hyphae penetrated the rootlet epidermis and progressed towards the rootlet cortical area by intercellular growth (indicated by arrows) and it was not associated with the host cells alteration or cells wall digestion (B-E).

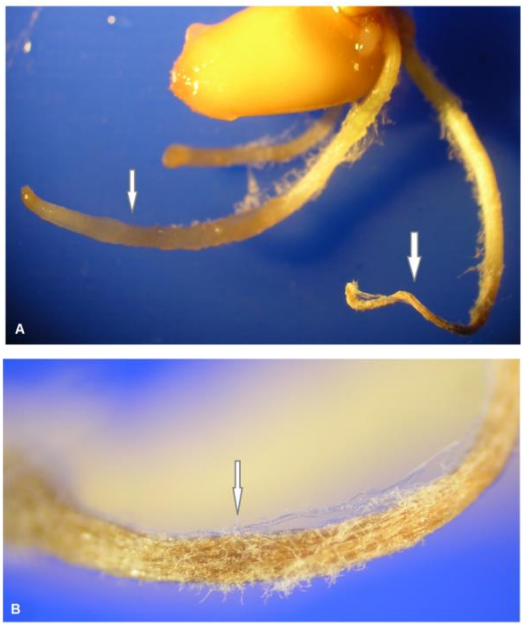

Fig 4. Necrotic regions (indicated by arrows) developed in the inner layer of emerged rootlet from *T. hamatum* T.614-inoculated seeds (**A** and **B**).

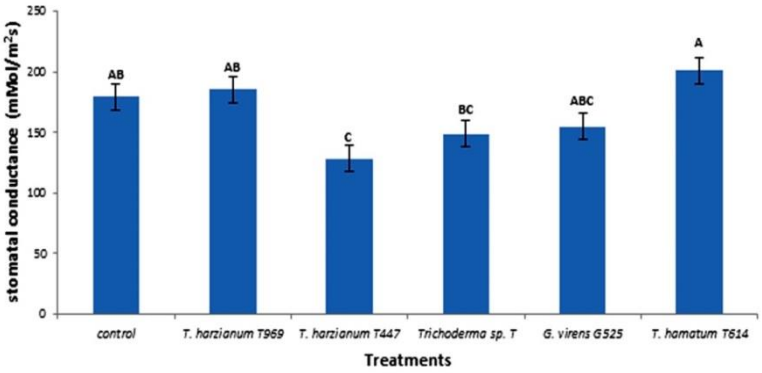

Fig 5. Effect of *Trichoderma* isolates on wheat stomatal conductance (The main effect of *Trichoderma* isolates). Data are expressed as means of stomatal conductance (mMol/m^2s) and values are average of 8 blocks. Means with the same letter are not significantly different (P≥0.05) by Duncan's Multiple Range Test for variable.

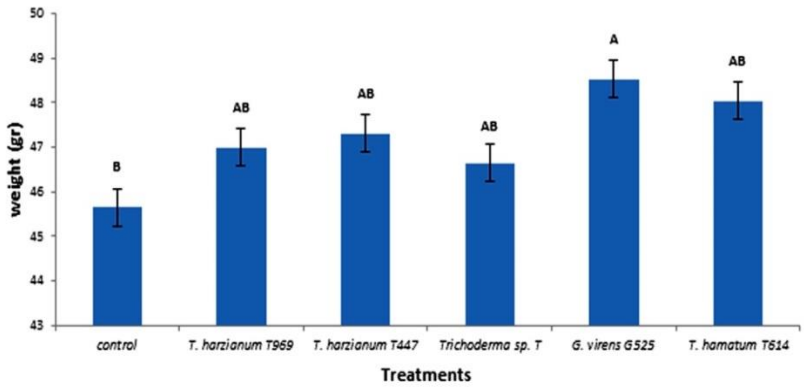

Fig 6. Effect of *Trichoderma* isolates on wheat grain weight (The main effect of *Trichoderma* isolates). Data are expressed as means of 1000 grains weight (gr) and values are average of 8 blocks. Means with the same letter are not significantly different (P≥0.05) by Duncan's Multiple Range Test for variable.

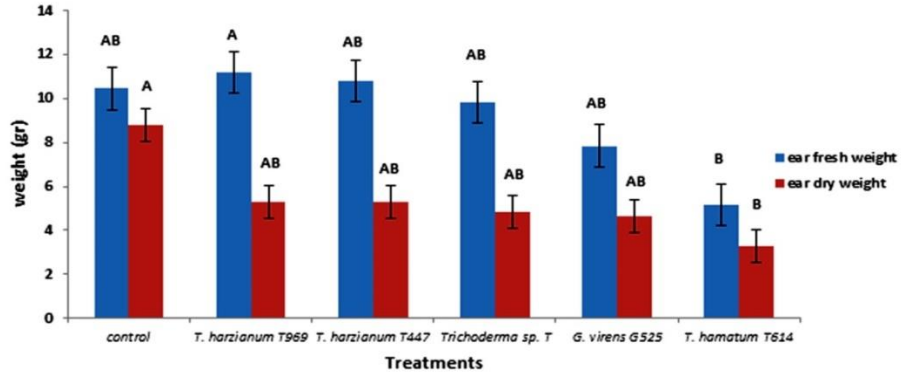

Fig 7. Effect of *Trichoderma* isolates on wheat ear fresh and dry (The main effect of *Trichoderma* isolates). Data are expressed of the average of 8 blocks. Means with the same letter are not significantly different (P≥0.05) by Duncan's Multiple Range Test for variable.

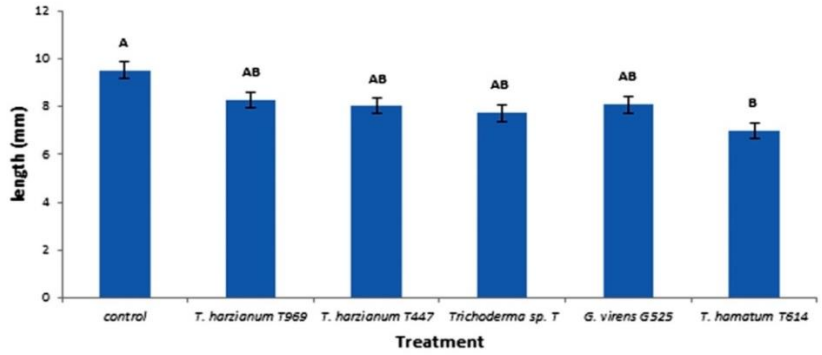

Fig 8. Effect of *Trichoderma* isolates on wheat ear length (The main effect of *Trichoderma* isolates). Data are expressed as means of ear length (mm) and values are average of 8 blocks. Means with the same letter are not significantly different (P≥0.05) by Duncan's Multiple Range Test for variable.

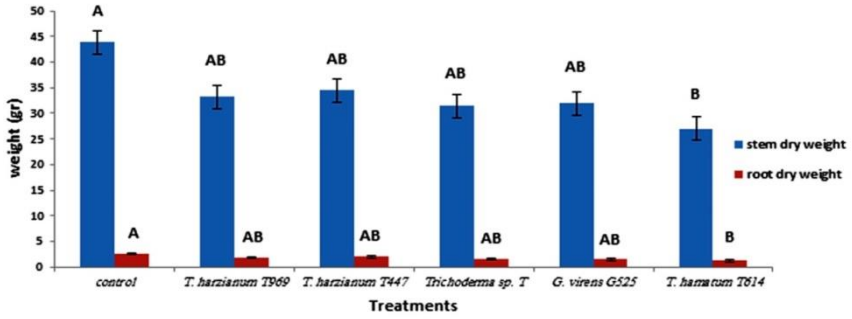

Fig 9. Effect of *Trichoderma* isolates on wheat stem and root dry weight (The main effect of *Trichoderma* isolates). Data are expressed of the average of 8 blocks. Means with the same letter are not significantly different (P≥0.05) by Duncan's Multiple Range Test for variable.

significant improvement in other measured parameters was seen for the *Trichoderma*-treated wheat plants.

Discussion

In the recent years, certain strains of *Trichoderma* have been demonstrated to be effective as biostimulants on plants growth and development and in a wide variety of plants (Harman et al., 2004; Bhardwaj et al., 2014). Some strains/isolates of *Trichoderma* may have better interaction with certain plant species, which can be called "*Trichoderma* species/isolate affinity" with some plant species. *Trichoderma* rhizosphere-competent strains have also been shown beneficial in plant growth and development. Several likely mechanisms have been suggested to explain this phenomenon such as the ability of mineral solubilization and increasing plant nutrient uptake (Altomare et al., 1999). Evidences also suggest that *Trichoderma* produces plant growth regulatory material and phytohormone such as Indol acetic acid and their analogous (Vinale et al., 2008a; 2012), vitamins, and enzyme leading to stronger root and shoot growth, and organic acids in rhizosphere such as gluconic, citric, and/or fumaric acids that decrease soil pH (Harman et al., 2004). Therefore, exploration and using plant growth and development promoting *Trichoderma* species/isolates may be helpful in improving and achieving the sustainable and eco-

friendly agriculture. Seed germination and seedling vigor mainly depend on seed germination rate, rapidity of root elongation and development during seed germination, and root and shoot fresh and dry weight. There are several mechanisms by which *Trichoderma* influences the seed germination and seedling vigor (Doni et al., 2014b) by betterment of the parameters which include but are not limited only to secretion of the seed germination stimulating factors and phytohormone (Zheng and Shetty, 2000; Clear and Valic, 2005) as well as enzymes involved in nutrition absorption (Jiang et al., 2011). As questioned *Trichoderma* species/isolates in improving seed germination, In this study, we observed that *T. harzianum* T969 non-sterilized culture filtrate significantly increased wheat seed germination rate but heat-sterilized culture filtrate did not have any effects. This observation suggests that the influent metabolites are heat-sensitive and become degraded by autoclaving. As demonstrated by the others, the effect of the *Trichoderma* specie/isolates secreted compounds in seed germination and seedling vigor depends not only on the *Trichoderma* species/isolate (as what we see in this study) and the type of metabolite(s) but also the plant species responses to the *Trichoderma* secreted metabolites are important (Gupta and Sharma, 1995; Celar and Valic, 2005; Vinale et al., 2012). Some strains/isolates of *Trichoderma* may have better interaction with certain plant species, which can be called "*Trichoderma* species/isolate affinity" with some plant species. In this sense, while culture filtrate of some *Trichoderma* species/isolates has been reported as stimulant of the seed germination in some plant species (Doni et al., 2014b), some other reports have described seed germination inhibitory effect of other *Trichoderma* species/isolates (Hajieghrari, 2010). Also, the observed result of *Trichoderma* cultural filtrate in seed germination and seedling vigor indicative far from to the observed result in introducing the Trichoderma species/ isolate to the spermosphere and rhizosphere. Notably, *T. harzianum* T969-fortified wheat seeds showed significant increase in germination rate. *T. harzianum* T969 also colonized the emerged rootlet surface, though the colonization was found to be superficially without penetrating in the tissue and injuring seedling rootlet. It seems that Environmental conditions especially in the colonized rhizosphere are the most important factors in what the plant root-*Trichoderma* interaction will result in. The capability of *Trichoderma* to colonize the spermosphere and rhizosphere of a plant root especially in root tip region is an important factor in its effectiveness not only in seed germination and promoting seedling growth but also in protection of the plant from soil-borne pathogenic fungi (Howell, 2003). Although we found that other *Trichoderma* species/isolates also were able to grow and colonize on the entire rootlet surface superficially, some of *Trichoderma* strains colonized only local sites on the roots. For eliciting increased growth response, *Trichoderma* must be initially able to establish an interaction with the plant root system. Colonization, surviving and developing of *Trichoderma* isolates in seed spermosphere and seedling rhizosphere mainly depend on certain compounds in the seed and seedling root exudates such as sugar and other carbohydrates, amino acids and organic acids (Howell, 2003) that lead to root colonization. For example, the highly hydrated polysaccharides of root-secreted mucosal layer and root-derived mono and disaccharides especially root-secreted sucrose are important energy sources that induce *Trichoderma* growth in the rhizosphere (Hermosa et al., 2012). We observed that *T. hamatum* T.614 was able to penetrate the rootlet epidermis and outer cortex and develop in the inner tissue leading to some necrotic region in wheat rootlet tissue. This observation did not surprise us since some reports have already highlighted the ability of some *Trichoderma* isolates to penetrate the root epidermis and colonize mostly through the intercellular spaces with or without causing extensive damage (Yedidia et al., 1999; Hajieghrari, 2010) evolving into a symbiotic rather than a parasitic microorganism. Such symbiotic *Trichoderma*-plant interaction may lead to direct molecular cross-talk resulting in expression certain genes involved in the promotion of the plant growth and/or in the plant innate defense against plant pathogenic microbes (Vinale et al., 2008). However, in some environmental conditions the symbiotic interaction may lean to pathogenic. The growth and biological activity of *Trichoderma* in the culture media could alter the chemical content and physical characteristics of the media. Na^+ and K^+ content and EC and pH in the culture media were modified. This modification in the growth medium is important since that root colonization and growth on root system as by *Trichoderma* may result in chemical changes in rhizosphere root system environment. This may result in increment of the insoluble compounds and availability of micronutrients as well as improving in and therefore increased plant nutrient uptake which can help the plant to grow more rapidly and it also enhances plant greenness which results in higher photosynthetic rate. All of these effects lead to will result in increase of the plant photosynthesis resulting in increase of and also carbohydrate metabolism and starch accumulation leading to increased energy availability required for enhancing growth response. The root-*Trichoderma* interaction establishment and effect of such interaction on plant growth and development as well as its influence on protection of the plant against plant pathogenic fungi in experimental conditions in laboratory are far away from the natural condition in the field (Hajieghrari et al., 2008). It seems that the soil chemical, physical and biological conditions in the field such as type of soil, soil micro flora and soil pH as well as watering and fertilizer regimes (Kredics et al., 2003) and other soil condition directly affect the receptivity of the plant to the growth-promoting factors as well as and also influences the Trichoderma production of some secondary metabolites and enzymes by *Trichoderma* having which has the most important role in what consequences will be occurred occur in the rhizosphere environment. These conditions have diverse efficacy on different *Trichoderma* species/isolates in confronting with a specified plant.

It is well known that biotic and abiotic environment can affect both the plant and *Trichoderma* in interaction with each other and with other plant pathogens. A better understanding of these interactions and the activity of the *Trichoderma* isolates/species in promoting plant growth can be achieved in field conditions. As we observed in the field experiments, the growth parameters response to the diverse employed *Trichoderma* species/isolates was different. *T. harzianum* T.447 had the most significant effect on decreasing stomatal conductivity in the wheat plants compared with other isolates and also the control. It is widely known that stomata are able to sense the changes that occur in the plant surrounding environment and they modify the effect of environmental conditions on plant physiology by controlling the transduction of water and CO_2, tuning the metabolism and environment. Lower stomata conductivity leads to lower plant water loss through stomata and on the other hand limited carbon gain and lower photosynthesis. In this case, some *Trichoderma* species/isolates may cause stress in the plant, leading to changes in plant metabolites

especially plant hormones which affect the stomata conductivity. Lower stomata conductivity also can affect the plant photosynthesis. However, significant reduction was not observed in the chlorophyll content of the *Trichoderma* fortified plants. Although *Trichoderma* isolates did not affect the wheat plants growth parameters, they increased 1000 grain weight in treated plants. *G. virens* G.525 inoculated plants showed more increase in 1000 grain weight, resulting in better quantitative improvement in the crop productivity and yield.

Materials and Methods

Seeds source

Wheat cv. Moghan 3 seeds were used in the experiments. The seeds were obtained from Seed and Plant Certification and Registration Institute, Karaj, Iran. Seeds were disinfected by immersion in 0.5% hypochlorite sodium (NaClO) for 5 min before being rinsed and washed thoroughly in sterile distilled water thrice in a laminar air flow cabinet, placed on a sterile blotting paper in sterile dishes for further uses.

Trichoderma isolates

Four Trichoderma isolates were obtained from the collection of *Trichoderma* spp. isolates in Iranian Research Institute of Plant Protection (IRIPP), Tehran including *T. harzianum* T 969, *T. harzianum* T 447, *T. hamatum* T 614, and *Gliocladium virens* G525. An isolate (*Trichoderma sp.* isolate T) was isolated from a wheat field in Moghan, Iran. The isolates were grown and maintained on Potato Dextrose Agar (PDA) plates and stored at 4 ºC.

Preparation of the spore suspension

Five millimeter diameter mycelia discs were cut from the margin of 7-day old PDA culture of each isolate and from an uncultured PDA plate and were centrally placed on 100 mL PDA in 250 mL conical flasks. After incubation at 25°C for 14 days, 20 mL sterile double distilled water was added to each flask and the flasks were shaken at 80 rpm for 30 min. The concentration of the spores in collected water was measured using haemocytometer and it was adjusted to 10^6 – 10^7 spores per milliliter in final spore suspension.

Preparation of the culture filtrate

Five discs of mycelia agar plugs (5 mm diameter) cut from the margin of 7-day old colony of each isolate were inoculated into 100 mL sterilized Potato Dextrose Broth (PDB) and incubated at 25°C for 14 days under constant shaking at 100 rpm. For controls, one of the medium flasks was inoculated by sterile distilled water and another one by discs cut from uncultured PDA plate. After centrifugation of the broths, they were filtered through Millipore filter for removing mycelia mats and then passed through 0.2 µm membrane filter (FP30/0.2 CA-S, Schleicher and Schuell MicroScience GmbH) for removing spores and mycelia mats. One half of the cultural filtrate was autoclaved at 121°C for 20 min.

Preparation of the seeds

The seeds were surface disinfected by immersion in 0.5% bleach solution for 3 min then were rinsed and washed in sterile distilled water thrice and air-dried in a laminar air flow cabinet.

Seeds treatment and germination assay

One hundred seeds were soaked in 10 mL spore suspension of each isolate and were placed on a sterile blotting paper inside a sterilized Petri dish. One hundred seeds were also placed on a sterile blotting paper in a separate sterile dish and were watered with 10 mL the autoclaved and non-autoclaved of culture filtrates from each isolates. In each experimental set, two control treatment sets, 10 mL sterile distilled water and 10 mL non-inoculated culture filtrate, were conducted. The dishes were incubated at 25°C. For evaluation of seed germination rate, rapidity of root elongation during seed germination, shoot and rootlet elongation; percentage of seed germination, coleoptile and rootlet weight, and rootlet and coleoptile length of seedling was measured after 48 and72 hours.

Field experiments and plant growth assay

The seeds were soaked in spore suspensions and spore-coated seeds were planted in the field. The parameters included the rootlet and shoot length, stem height and weight, root length and weight, total leaf area, head and tiller number, grains number and weight (1000 grain weight). The rootlet and shoot length were measured early tillering stage. Stem length and weight, root length and weight, and head and tiller number were measured at the early ripe stage. The values for total leaf area, head and tiller number, grains number and weight (1000 grain weight) were measured at maturity stages. For root length and weight measurement, the plants were uprooted and the roots were washed under running tap water to remove residual soil. Total chlorophyll content and stomatal conductance of the plants were measured using Chlorophyll Meter SPAD 502 (Konica Minolta Sensing Inc, Japan) and SC-1 Leaf Promoter (Decagon, USA) respectively at the early ripe stage. Also, Total leaf area was measured using LI-3100 Area Meter (Li-COR Biosciences, USA) at grain filling stage. For each measurement, five wheat plants per each row were used and the values are the average of the means of each row in the block.

Experimental design

In all laboratory experiments including seed germination assay, seedling and rootlet lengths, crown diameter and shoot and rootlet fresh weight study, each treatment was set up in four replications (four Petri dishes) forming a completely randomized design (CRD). In the field part, two experiments were conducted at two different locations simultaneously in the spring of 2014 in Moghan, northwest of Iran. Both field experiments were carried out in four blocks (replications) designed in randomized complete block design (RCBD). Each block contained 15 rows and each row was 5 m long and received one seed per 5 cm.

Statistical analysis

The means of the parameters from four replications in all laboratory and field experiments were statistically analyzed by ANOVA followed by Duncan's Multiple Range Test for variable at 1% and 5% significant level using SAS software (SAS Institute Inc., Cary, NC, USA) to separate the means.

Conclusion

In this study, *Trichoderma* non-sterilized metabolites had significant effect on wheat (variety Moghan 3) seed germination and seedling vigor, but heat-sterilized metabolites did not have any effects. Significant effects on seed germination and seedling vigor was also observed in the seeds inoculated by *Trichoderma* spores. In the field, we observed marked effects of the *Trichoderma* isolates on parameters such as leaves stomata conductivity, 1000 grain weight, ear fresh and dry weights, ear length and stem and root dry weight, but no effect on other evaluated parameters was observed. In overall, some isolates were able to affect growth parameters in aerial organs by colonizing the wheat rhizosphere. Screening of *Trichoderma* isolates that not only are able to control soil pathogenic fungi but also are able to promote some beneficial growth parameters may help the plant standing in better growth position.

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