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How May *Trichoderma* Application Affect Vegetative and Qualitative Traits in Tulip "Darwin Hybride" Cultivar

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ABSTRACT

To study the effect of *Trichoderma harzianum Bi* on qualitative and quantitative traits of tulip including stem length and diameter, bud diameter, petal length, bulb perimeter and bulblet number, an experiment based on completely randomized design with 8 replications were completed in situ. Main media mixture was 40% of coco peat+ 40 of field soil+ 20% of perlite. Treatments included enriched coco peat with *trichoderma* in different concentrations (0, 20, 50 and 100%) of total coco peat. Results showed that 100 and 50% of enriched coco peat significantly increased stem length and diameter. These treatments also had an effect on bud diameter significantly. Petal length was also affected by 100% of enriched coco peat treatment in comparison to control. All treatments (20, 50 and 100%) had a significant effect on bulblet appearance in both mature and immature bulbs. Mature bulb perimeter was significantly affected by all treatments although immature bulb perimeter was only affected by 50 and 100% treatments. Flower bud formation in immature bulbs which was treated with 50 and 100% of enriched coco peat increased significantly in comparison to control. Totally it seems that trichoderma increases tulip cut flower quality traits and induces maturity in immature bulbs.

Keywords: culture medium, enriched coco peat, flower bud diameter, bulb quality.

INTRODUCTION

Trichoderma harzianum is a saprophytic fungus which is used generally as a biological control agent against a wide range of economically important aerial and soilborne plant pathogens (Papavizas,1985). *Trichoderma* spp. have been extensively studied as potential biocontrol agents (e.g. Lynch 1990; Papavizas 1992). However, some studies have also shown that it can stimulate the growth of a number of vegetable and bedding plant crops (e.g. Baker 1989; Lynch et al. 1991a,b). Lynch et al. (1991a,b) investigated the effect of *Trichoderma sp.* on the growth of lettuce, and its ability to control damping off diseases caused by *Rhizoctonia solani* and *Pythium ultimum*. They showed also that a number of *Trichoderma* strains had a direct effect on lettuce establishment and it's growth ratio in absence of pathogens. They found that the fungal treatments of seed reduced the emergence time of seedlings compared to the controls. From their results, and those of Ousley et al. (1994), they concluded that some *Trichoderma* strains have the potential to consistently increase plant growth (Lynch et al. 1991a) and influence it's phenology.

Various species of *Trichoderma* were also effective in the promotion of growth and yield in various crops (Bal and Altintas, 2006a). *T. harzianum* and *T.virens* promoted growth of cucumber and cotton seedlings (Hanson, 2000; Poldma et al., 2000; Yedidia et al., 2001). Root and shoot growth of sweet corn was considerably increased (Bjorkman et al., 1998). Cucumber, bell pepper and strawberry yields were increased significantly following the application of *T. harzianum* in the root zone (Poldma et al., 2002; Altintas and Bal, 2005; Bal and Altintas, 2006b; Elad et al., 2006). However, application of *Trichoderma spp.* was not conducive to increased yields in tomato (Bal and Altintas, 2006c). As for onion, yield and quality characteristics were not enhanced by the application of *Trichoderma* spp. (Poldma et al., 2001). *Trichoderma* species can improve plant growth and development (Chang et al., 1986; De Souza et al.,2008; Gravel et al., 2007; Windham et al., 1986). Growth stimulation is evidenced by increases in biomass, productivity, stress resistance and increased nutrient absorption (Hoyos-Carvajal,2009).

The plant benefits by the presence of *Trichoderma spp.*, suggesting an interaction as avirulent symbionts (Howell et al. 2000; Harman et al., 2004; Yedidia et al.,1999, 2000). *Trichoderma spp.* can also produce metabolites with activities analogous to plant hormones (Cutler et al., 1989, 1991).

In this study we aimed to find if *Trichoderma spp.* had additional and promoting effects on vegetative and qualitative traits of tulip bulbs and cut flowers.

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MATERIALS AND METHODS

Experimntal design and data analysis

To assess the effect of *Trichoderma harzianum Bi* on qualitative and quantitative traits of tulip, an experiment was performed in situ using completely randomized design with 4 treatments and 8 replications. The data was analysed with MSTATC software. Duncan's multiple range test in P<1% and P<5% used for grouping and comparing the meanss.

Inoculum preparation

T. harzianum Bi obtained from Ferdowsi University of Mashhad fungi collection. The isolate Bi was cultured on PDA and incubated at 25˚C for 5 days. Four discs of 1.5 cm diameter were cut from the margin of Trichoderma colony and added to 1 lit Erlenmeyer containing 250 g. wheat grain boiled and autoclaved for 30 minutes. They kept for 15 days at 25˚C till covering completely the grain by trichoderma. The grains then were mixed at the rate of 10 % (V.V) with peat, autoclaved in polyethylene bags (resistant to high temp.) for 30 mins, and placed at 25˚C ± 5 in laboratory condition. 10 days later, when the peat was covered by Trichoderma, the contents of bags were used as Trichoderma inoculums.

Culture medium

The prepared inoculums were added to the main potting mixture (40% CoCo peat+ 40% fertile soil+ 20% perlite) at the rate of 0 (as control), 20, 50 and 100% of used coco peat. No manure or nutritional solutions were used during this survey. Weed control and irrigation was equal in all treatments.

Bulb preparation

Tulip bulbs *"Darwin Hybride"* cultivar were supplied commercially. Then 32 mature bulbs with 12±0.5 cm perimeter and 48 immature bulbs with 4±0.3 cm perimeter were selected. Bulbs were disinfected using sodium hypochlorite then desiccated, before sowing. Pots with 20 cm diameter were used for mature and with 15 cm diameter were used for immature bulbs. 2 mature and 3 immature bulbs were planted per pot.

Data collection

The data of when vegetative bud emerged on soil surface and flower bud appeared were collected in February. Stem length and diameter and flower bud diameter were measured when 1/3 flower bud was colored. Petal length measurement was done immediately after bloom. After vegetative period in midsummer, bulbs were emitted and bulblet appearance quantity in mature bulbs and perimeter of mature and immature bulbs were measured. Then immature bulbs were sectioned and flower bud situation was studied.

RESULTS

Analysis of variance for mean square of studied traits indicate that among different *trichoderma* concentrations applied on mature bulbs, significant differences were observed for stem length and diameter, bud diameter, bulblet number and bulb perimeter in P<1% while for petal length differences between treatments were significant in P<5% (Table.1).

Table 1. Mean square analysis of variance for studied traits in mature tulip bulbs treated with different *T.harzianum Bi.* concentrations.

C.V.	**d.f**	**Bulb perimeter**	**Bulblet number**	**Petal length**	**Bud diameter**	**Stem diameter**	**Stem length**
Trichoderma	3	7.838**	9.417**	1.263*	0.332**	0.107**	323.914**
Error	28	0.85	0.431	0.316	0.022	0.009	*10.412*

:significant in P<1%** , :significant in P<5%*

Results showed that as *Trichoderma* concentration increases, the stem length increases also. In the highest concentration of *trichoderma* two fold increase in comparison to control is obvious. Maximum stem length were observed in Treatment with 100% of enriched coco peat(26.46 cm) which was higher than other treatments statistically (Table.2).

Significant increase in stem diameter occurs when treatment concentration increased from 50 % to 100%.
Maximum increase in bud diameter and petal length were in 100 concentration of enriched coco peat although there were not any significant difference between 50 and 100% treatments for these two traits (Table.2).
For bulb perimeter , maximum increase in mature bulbs occur in 100% treatment while between 100 and 50 % treatments differences were not significant but difference between 20 and 50% treatments was significant (Table.2).

Table 2. Effect of *T.harzianum Bi.* on studied traits in mature bulbs of tulip "*Darwin hybride*"cultivar.

Eiched cocopeat treatments (%)	**Bulb perimeter**	**Bulblet number**	**Petal length**	**Bud diameter**	**Stem diameter**	**Stem length**
100	6.125 [a]	11.940 [a]	5.525 [a]	2.408 [a]	0.775 [a]	26.46 [a]
50	5.125 [ab]	11.680 [a]	5.137 [ab]	2.332 [a]	0.647 [b]	21.59 [b]
20	4.750 [b] 4.750 [b]	10.730 [b]	4.700 [b]	2.017 [b]	0.551 [c]	14.89 [c]
control	3.500 [c]	9.762 [c]	4.700 [b]	2.025 [b]	0.516 [c]	*12.50 [c]*

Means with different letter have significant difference at P<5%, Duncan's Multiple range test.

Bulblet appearance number was in its maximum with 100% treatment. However all treatments were significantly higher than control but there were not any significant differences between 100 and 50% treatments and 50 and 20% treatments (Table.2).
Immature bulbs also had significant differences in P<1% for bulblet number, bulb perimeter and flower bud formation in all treatments (Table.3).

Table 3. Analysis of variance of the effect of different inoculums percentage of *T.harzianum Bi.* On immature bulbs

C.V.	d.f	Flower bud formation	Bulb perimeter	Bulblet Number
Trichoderma	3	110.677 **	3.895 **	3.615**
Error	28	27.183	0.423	0.531

N.S.: non- significant

However there were not any significant difference between 20, 50 and 100 % of *trichoderma* for bulblet number, but all treatment was successful to increase bulblet number significantly in comparison with control (Table.4). Increase in *T.harzianum Bi. c*oncentration make significant increase in immature bulb perimeter , where minimum perimeter occur in control and maximum perimeter tide in 100% treatment (Table.4).
Flower bud formation significantly increased when concentration of *T.harzianum Bi* increased from 20% to 50% but no more increase were observed in bud formation when *trichoderma* increment resumed (Table.4).

Table 4. Effect of *T. harzianum Bi.* Inoculum percent on studied traits of immature bulbs of tulip "*Darwin hybride*"cultivar.

Eiched cocopeat treatments (%)	**Flower bud formation**	**Bulb perimeter**	**Bulblet number**
100	9.375 [a]	8.762 [a]	3.50 [a]
50	7.813 [ab]	7.988 [b]	2.875 [a]
20	3.125 [bc]	7.438 [bc]	2.875 [a]
control	1.563 [c]	7.188 [c]	*1.875 [b]*

Means with different letter have significant difference at P<5%, Duncan's Multiple range test.

DISCUSSION

In this study *T. harzianum Bi* application to culture medium was statistically effective on promotion of vegetative and qualitative traits of tulip *"Darwin hybride"* cultivar. Ously and et al.,(1994) delineated that some *Trichoderma* strain had inductive effects on growth of *calendula* and *petunia*. In another study dubsky (2002) declare that *T.harzianum* inoculation to peat was significantly impressive on growth and flowering of poinsettia and cyclamen.

Several mechanisms, by which *Trichoderma spp.* influences plant development were suggested, such as production of growth hormones (Windham et al., 1986), solubilization of insolubleminor nutrients in soil (Altomare et al., 1999) and increased uptake and translocation of less-available minerals (Baker, 1989; Inbar et al., 1994; Kleifeld and Chet, 1992). Uptake of certain minerals, such as P and N, is of key importance considering their role in plant growth (Johansen, 1999; Kim et al., 1997). Promotion of growth and yield by *Trichoderma* spp. may also be a result of increased root area allowing the roots to explore larger volumes of soil to access nutrients, and increased solubility of insoluble compounds as well as increased availability of micronutrients (Altomare et al., 1999; Yedidia et al., 2001). However, initially *Trichoderma* must be able to establish an interaction with the root system. The ability of a *Trichoderma* species to colonize the root system of a plant depends also on the plant species.

The increased growth response of plants caused by *T.harzianum* depends on the ability of the fungus to survive and develop in the rhizosphere (Kleifield and Chet 1992). A possible mechanism for increased plant growth is an increase in nutrient transfer from soil to root, which is supported by the fact that *Trichoderma* can colonize the interior of roots (Kleifield and Chet 1992).

Increasing effects of *T.harzianum* on plant growth and yield was suggested to be more pronounced in soils relatively poor in nutrients (Rabeendran et al., 2000). Availability of water in the soil may play an important role in facilitating establishment and effectiveness of *Trichoderma* in the soil (Altintas, 2007).

The production of plant growth hormones or analogues is another mechanism by which strains of *Trichoderma* can enhance plant growth. 162 species of fungi have been reported to produce auxins, which are key hormones affecting plant growth and development that can be produced by fungi in both symbiotic and pathogenic interactions with plants (Gravel et al., 2007; Losane and Kumar, 1992; Patten and Glick, 2002; Shayakhmetov, 2001). In a study, seed germination of cucumber increased by application of *Trichoderma* and this increment may be due to hormonal factors secretion like Gibrellins, Auxins or ethylene (Akter, et al., 2007).

In addition to having a stimulating effect on plant growth, exogenous IAA in the rhizosphere can also have a detrimental effect on the elongation of roots over a wide range of concentrations. Such an effect has been associated with an increase in the level of ethylene in the plant (Glick et al., 1997, 1998). IAA can increase the activity of ACC synthase, which catalyses the conversion of S-adenosyl methionine to ACC, the precursor of ethylene in the plant (Kende, 1993). The plant growth stimulation reported in Gravel,2007 is, most likely, the synergic result of numerous modes of action exhibited by *T.atroviride*, including a regulation in the concentration of IAA in the rhizosphere and a regulation of the concentration of ethylene within the roots.

The results reported by vinale et al., 2008, clearly indicated that some *Trichoderma* secondary metabolites are directly involved in the Trichoderma-plant interactions, and particularly that the compound 6PP may be considered to act as an auxin-like compound and/or may act as an auxin inducer. The identification of new molecular effectors may support the application of new biopesticides and biofertilizers based on *Trichoderma* metabolites to be used instead of the living microbes as elicitors of plant defense mechanisms and plant growth stimulants(Vinale, et al., 2008).

According to the results of privous studies conducted over the past 5 to 10 years as well as (Harman, 2006) and results of *T.harzianum* on traits studied in this experiment, it can be concluded that promotional effects of *trichoderma* may be due to these reasons:

- Control of root and foliar pathogens
- Induced resistance
- Biological control of diseases by direct attack of plant pathogenic fungi
- Changes in the microfloral composition on roots
- Enhanced nutrient uptake,(not limited to nitrogen)
- Enhanced solubilization of soil nutrients
- Enhanced root development

- Increased root hair formation
- Deeper rooting

CONCLUSIONS

In conclusion, some *Trichoderma* spp. can be a determining factor impacting on the microbial community in the rhizosphere to enhance or even inhibit plant growth, and occasionally establishing a positive interaction within plant roots as an endophyte. The latter association may be the most predictive for the selection of specific strains that can be used as bioinculants to improve crop health and productivity. Direct plant-fungi interactions induce changes in both the fungus and plant transcriptomes affecting genes that regulate plant physiology including growth and plant defenses (Harman, 2006; Woo et al., 2006; hoyos-carvajal,2009). In this study we found *Trichoderma harzianum* strain *Bi* that could stimulate both early stages of growth (flower bud formation in immature bulblets) and late stages of growth (stem, petal and mature bulb properties).

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