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Does *Trichoderma harzianum* Really Increase Growth Parameters in Plants?

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Abstract: To study the effect of *Trichoderma harzianum* Bi on qualitative and quantitative traits of tulip and gladioluss 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. For gladiolus results showed that 50 and 100% of enriched coco peat significantly increase stem diameter in compare with 20% and control but there was not any significant difference between 100 and 50% treatments. 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. None of the treatments applied was not able to significantly change (increase or decrease) stem length, inflorescence floret number, Postharvest opened floret number and leaf length of main and lateral buds in gladiolus corms. The data obtained from the experiment show that trichoderma increases polianthes cut flowers qualitative and quantitative traits.

Key words: Culture medium, enriched coco peat, flower bud diameter, bulb quality, polianthes, Iran

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* sp. have been extensively studied as potential biocontrol agents (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 (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 also showed that a number of trichoderma strains had a direct effect on lettuce establishment and it is 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 its 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*., 1981). However, application of *Trichoderma* sp. 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* sp. (Poldma *et al*., 2001).

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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 *et al*., 2009).

The plant benefits by the presence of *Trichoderma* sp., suggesting an interaction as avirulent symbionts (Howell *et al*., 2000; Harman *et al*., 2004; Yedidia *et al*., 1999, 2001). *Trichoderma* sp. can also produce metabolites with activities analogous to plant hormones (Cutler *et al*., 1989, 1991).

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

MATERIALS AND METHODS

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 L Erlenmeyer containing 250 g. Wheat grain boiled and autoclaved for 30 min. 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 min and placed at 25°C±5 in laboratory condition. About 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: The 32 mature bulbs with 12±0.5 cm perimeter and 48 immature bulbs with 4±0.3 cm perimeter of tulips and 28 corms of gladiolus with 14±0.5 cm perimeter were selected.

Bulbs and corms 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. The two mature and three immature bulbs were planted per pot.

Experimntal design and data analysis: To assess the effect of *Trichoderma harzianum* Bi on qualitative and quantitative traits of tulip, 2 experiments (one for tulip and one for gladiolus) were 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 means.

RESULTS AND DISCUSSION

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).

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 50-100%. 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%

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

CV	df	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	0.850	0.431	0.316	0.022	0.009	10.412

*Significant in $p<5\%$; **Significant in $p<1\%$

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

Eiched coco peat 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}	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 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).

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). 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 concentration 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 20-50% but no more increase were observed in bud formation when trichoderma increment resumed (Table 4).

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

CV	df	Flower bud formation	Bulb perimeter	Bulblet number
Trichoderma	3	110.677**	3.895**	3.615**
Error	28	27.183	0.423	0.531

**Non significant

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

Eiched coco peat treatments (%)	Flower bud formation	Bulb perimeter	Bulblet number
100	9.375^{a}	8.762^{a}	3.500^{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

Results also showed that 50 and 100% of enriched coco peat significantly increase stem diameter in compare with 20% and control but there was not any significant difference between 100 and 50% treatments in gladiolus (Table 5). The treatments was not able to significantly change (increase or decrease) stem length, inflorescence floret number, postharvest opened floret number and leaf length of main and lateral buds in gladiolus corms (Table 6).

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 *et al.* (1994) delineated that some trichoderma strain had inductive effects on growth of calendula and petunia. In another study, Dubsky *et al.* (2002) declare that *T. harzianum* inoculation to peat was significantly impressive on growth and flowering of poinsettia and cyclamen.

Several mechanisms by which *Trichoderma* sp. 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; Kleifield 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* sp. 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

Table 5: Mean square analysis of variance for the studied traits in gladiolus corms treated with different *T. harzianum* Bi concentrations

CV	df	Stem length	Stem diameter	Floret number	Number of opened florets in postharvest	Leaf length
Trichoderma	3	936.69^{NS}	4.149**	2.988^{NS}	2.095^{ns}	9507.412^{NS}
Error	24	533.21	0.650	2.400	1.960	7527.160

**Significant at p<1%, *Significant at p<5%; NS: Non Significant

Table 6: The effect of *T. harzianum Bi* on the studied traits in corms of gladiolus

Eiched coco peat treatments (%)	Stem length	Stem diameter	Floret number	No. of opened florets in postharvest	Leaf length
100	102.2^{a}	4.36^{a}	12.57^{a}	10.29^{a}	279.3^{a}
50	102.3^{a}	4.22^{a}	12.29^{a}	9.71^{a}	295.7^{a}
20	83.79^{a}	3.35^{b}	11.14^{a}	9.14^{a}	327.2^{a}
Control	80.86^{a}	2.72^{b}	12.43^{a}	10.29^{a}	362.8^{a}

Means with different letter in the same column have significant difference at p<5%, Duncan's multiple range test 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 and Bal, 2008).

The production of plant growth hormones or analogues is another mechanism by which strains of trichoderma can enhance plant growth. About 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 *et al*. (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 5-10 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

CONCLUSION

In this study, some Trichoderma species 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 *et al*., 2009). In this study, the researchers 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) in tulips but it was not significantly effective in growth stimulation of gladiolus corms. This may happen because of different reactions in different species to *T. harzianum* Bi activities or also trichoderma could have transudation of some materials in the soil which may have allelopathic effects on gladiolus corms.

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