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Trichoderma harzianum and Fe Spray Improve Growth Properties of *Spathiphyllum* sp.

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Abstract

Effects of Fe and *Trichoderma harzianum* Bi strain on plant growth and development of *Spathiphyllum* were investigated. Experiments were carried out in an glasshouse and in pots filled with soil, perlite and coco peat (1:1:1) were used as the growing medium. Plant roots (seedlings with three leaves) were inoculated with *Trichoderma* (0 and 8% w/w) as media mixture. Fe spray (0, 0.75, 1.5, 3 g/L), was applied 3 times on a month interval after *Trichoderma* inoculation. Factorial experiment was conducted in a completely randomized design with 3 replications. After six months, the plants were sampled for growth comparisons. Based on results *Trichoderma* improved morphological characteristics ($P \leq 0.01$). There were differences between the untreated control and the treatments for all of the growth parameters with the exception of spathe area and number of flowers. Fe spray and intraction between *Trichoderma* and Fe significantly increased all morphological growth parameters with the exception of spathe area, leaf area and number of flowers. By applying *Terichoderma* sucker number (400%), leaf number (586%), sucker fresh weight (386 %) and sucker dry weight (583%) significantly increased compared with control. The data obtained from the experiment showed the potential of *Trichoderma* and Fe spray to enhance growth and development of *Spathiphyllum* sp. in greenhouse conditions.

Keywords: Bi strain, Fe, Growth characteristics, Potted plant.

INTRODUCTION

Trichoderma is a saprophytic fungus which is used generally as a biological control agent against a wide range of economically important aerial and soil-borne plant pathogens (Papavizas, 1985). The genus *Trichoderma* is cosmopolitan in soils and on decaying wood and vegetable matter. Species of *Trichoderma* are frequently dominant components of the soil microflora in widely varying habitats. This may be attributable to the diverse metabolic capability of *Trichoderma* species and their aggressively competitive nature. Strains of *Trichoderma* are rarely associated with diseases of living plants, although an aggressive strain of *Trichoderma* causes a significant disease of the commercial mushroom (Muthumeenakshi *et al*., 1998). *Trichoderma* species are cosmopolitan and abundant fungi in soil in a wide range of ecosystems and climatic zones. They are characterized by rapid growth, capability of utilizing diverse substrates and resistance to noxious chemicals (Klein and Eveleigh, 1998). *Trichoderma* species can improve plant growth and development (De Souza *et al.,* 2008; Gravel *et al.,* 2007). Growth stimulation is evidenced by increases in biomass, productivity, stress resistance and increased nutrient absorption (Hoyos-Carvajal *et al*., 2009). *In vitro* studies have shown that micronutrients and insoluble phosphates became soluble and available, therefore useful to the roots interacting with *Trichoderma* in the root zone (Altomare *et al*., 1999). Various species of *Trichoderma* were also effective in the promotion of growth and yield of various crops (Bal and Altintas, 2006). *T. harzianum* and *T. virens* promoted growth of cucumber, muskmelon and cotton seedlings (Hanson, 2000; Poldma *et al.*, 2000; Yedidia *et al.*, 2001, Kaveh *et al.,* 2011). Root and shoot growth of sweet corn were considerably increased by *Trichoderma* (Bjorkman *et al*., 1998). Several mechanisms, by which *Trichoderma* influences plant development were suggested, such as production of growth hormones (Windham *et al*., 1986), solubilization of insoluble minor nutrients in soil (Altomare *et al.,* 1999) and increased uptake and translocation of less-available minerals (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* 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). The increased growth response of plants caused by *Trichoderma* 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 *Trichoderma* 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., 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*., 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).

MATERIALS AND METHODS

An experiment was conducted in the greenhouse facilities at Ferdowsi University of Mashhad, Iran. Inoculum production of *T. harzianum* Bi was obtained from fungi collection of Ferdowsi University's plant protection department. The Bi strain was cultured on PDA and incubated at 25˚C for 7 days. Four discs of 1.5 cm diameter were cut from the margin of *Trichoderma* colony and added to wheat grain, autoclaved two times in polyethylene bags (resistant to high temperatures) for 45 minutes, and placed at 25˚C ± 5, in laboratory condition. Ten days later, when the peat was covered by *Trichoderma*, the contents of the bags were used as *Trichoderma* inoculums. Prepared inoculums were added to the main potting mixture (30% coco peat+ 40% fertile soil+ 30% perlite) at the rate of 0 and 8% of used medium.

Seedling preparation

Seedlings of *Spathiphyllum* were obtained from university greenhouse and were cultivated in pots with 10 cm in diameter.

Experimental Design and Data Analysis

To asses the effect of *Trichoderma* (Bi) and Fe spray on growth characteristics of *Spathiphyllum*, a factorial experiment was performed *in situ* using RCD with 8 treatments and three replications. The data was analyzed with JMP8 software. Tukey HSD was used for grouping and comparing the means.

RESULTS

Leaf count

Leaf number was showed to be significant with applying *Trichoderma* and Fe treatments. Minimum number of leaf (7) was observed in 0.75 g/L Fe, whereas maximum number (48) was observed in *Trichoderma* and 0.75 g/L Fe interaction. Rising Fe treatments showed no significant leaf number increase, but adding *Trichoderma* had a noticeable increase. It means that interaction effects were the most effective for leaf number in this experiment. Fe treatments up to 0.75 were observed simulative for growth and leaf number, but higher concentrations decreased this trait which could be considered as a toxic influence (Fig. 1).

Sucker count

By applying *Trichoderma* and Fe treatments, number of sucker was significantly increased ($p \leq 0.01$). Minimum (0) and maximun (4) number of sucker was observed in Fe treatments and in *Trichoderma* and 0.75 g/L Fe interaction, respectively. Rising Fe treatments showed no significant sucker number increase, but applying *Trichoderma* had a considerable increase. Generally interaction effects were the most effective in sucker number. Fe treatments up to 0.75 g/L were observed simulative for growth and sucker number, but higher concentrations decreased this trait which could be considered as a toxic influence (Fig. 2).

Sucker fresh weight

Sucker fresh weight was showed to be significant with applying *Trichoderma* and Fe treatments ($p \leq 0.01$). Minimum weight was observed for Fe (0 g/L) which had no significant differences with 1.5 g/L Fe. Maximum weight was related to *Trichoderma* and 0 g/L Fe interaction. It means

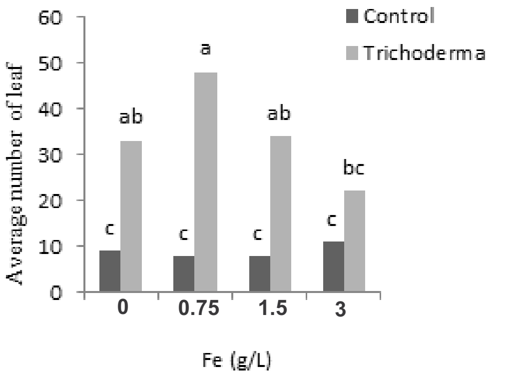

Fig. 1. Effect of interaction between *Trichoderma* and Fe on average number of leaf

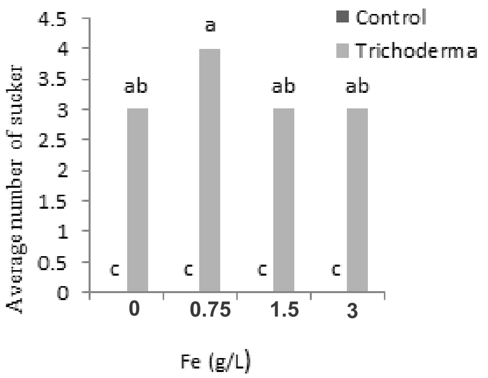

Fig. 2. Effect of interaction between *Trichoderma* and Fe on average number of sucker

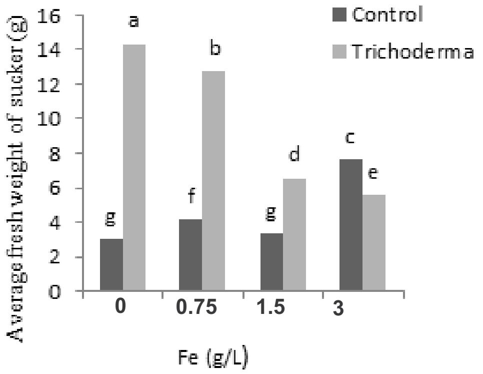

Fig. 3. Effect of interaction between *Trichoderma* and Fe on average fresh weight of sucker

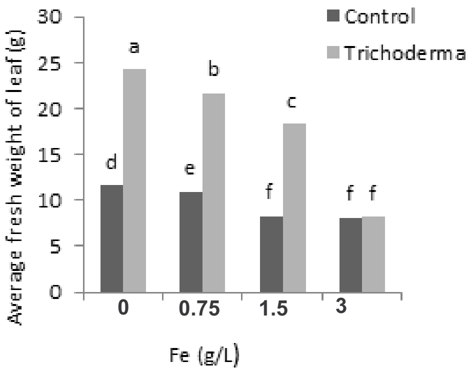

Fig. 4. Effect of interaction between *Trichoderma* and Fe on average fresh weight of leaf

that applying *Trichoderma* had a noticeable effect on sucker fresh weight increase with the exception of 3 g/L Fe. Increasing Fe levels, decreased average sucker fresh weight in *Trichoderma* treatments that seem to be due to the toxicity of high concentrations of Fe in plant (Fig. 3).

Leaf fresh weight

Leaf fresh weight was showed to be significant in the presence of *Trichoderma* and Fe treatments. The highest (24 g) weight was related to *Trichoderma* and 0 g/L Fe. While the lowest weight (7 g) was observed in 0 g/L Fe. It generally seem that Fe would not have any positive effect on this trait and with increasing concentration, decreases the average fresh weight of leaf. *Trichoderma* significantly increased leaf fresh weight. Interaction effects were less effective for this trait (Fig. 4).

Root fresh weight

Applying *Trichoderma* and Fe had a remarkable effect on root fresh weight ($p \leq 0.01$). Minimum fresh weight of root was observed in 3 g/lit Fe (8 g) and maximum weight was related to *Trichoderma* and 0/lit Fe (31 g). In Fe treatments with increasing level of Fe, decrease fresh weight of root. While, in *Trichoderma* and Fe interaction treatments, 0g/L Fe had a greater effect than others. This reduction in fresh weight in high concentrations could be due to a toxic influence (Fig. 5).

Flower fresh weight

Flower fresh weight was showed to be significant with applying *Trichoderma* and Fe treatments ($p \leq 0.01$). For this trait the most effective treatment was *Trichoderma* and 0 g/L Fe, which was significantly more than other treatments (10.8 g). Minimum flower fresh weight was observed in *Trichoderma* and 3 g/L Fe (2 g). Rising Fe treatments showed significant leaf number decrease and by adding *Trichoderma*, just 0 and 1.5 g/L Fe showed an increase. It means that interaction effects were less effective for this trait (Fig. 6).

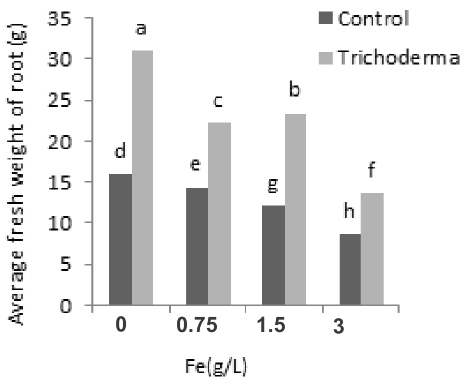

Fig. 5. Effect of interaction between *Trichoderma* and Fe on average fresh weight of root

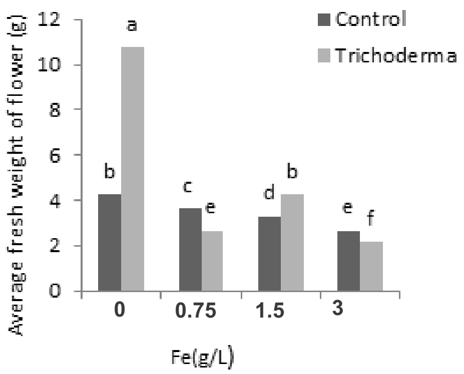

Fig. 6. Effect of interaction between *Trichoderma* and Fe on average fresh weight of flower

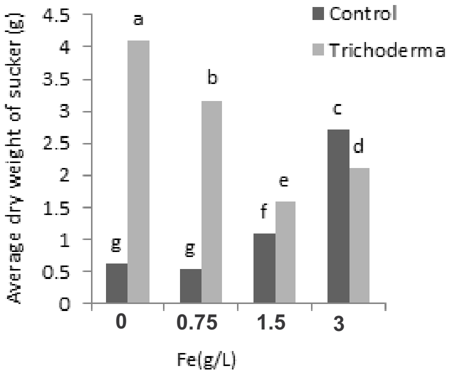

Fig. 7. Effect of interaction between *Trichoderma* and Fe on average dry weight of sucker

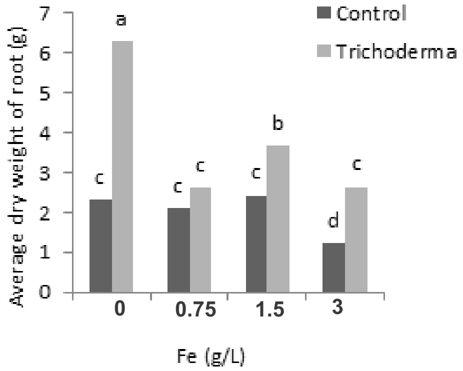

Fig. 8. Effect of interaction between *Trichoderma* and Fe on average dry weight of root

Sucker dry weight

Applying *Trichoderma* and Fe had a remarkable effect on dry weight of sucker. Maximum weight(4.1 g) was observed in *Trichoderma* and 0 g/L Fe interaction whereas minimum weight (0.5 g) was related to 0.75 g/L Fe. Rising Fe treatments showed significant sucker dry weight increase and adding *Trichoderma* had a noticeable increase except in 3 g/L. It means that interaction effects were the most effective for sucker dry weight generally. Fe treatments up to 3 were observed simulative for growth and dry weight of sucker (Fig. 7).

Root dry weight

Applying *Trichoderma* and Fe treatments, root dry weight was significantly increased.Minimum weight was observed in 3 g/L Fe (1.2 g), whereas maximum weight was related to *Trichoderma* and 0 g/L Fe interaction (6.15 g). Rising Fe treatments showed no significant weight increase, but adding *Trichoderma* had a noticeable increase in most treatments especially at 0 g/L Fe (37%). It means that interaction effects were the most effective for root dry weight in this experiment (Fig. 8).

Flower Dry Weight

Flower dry weight was showed to be significant in the presence of *Trichoderma* and Fe treatments. Dry weight of flower had the highest range in 0.75 g/L Fe (1.35 g) which had no significant differences with 1.5 g/L. Minimum weight was observed in *Trichoderma* and 3 g/L Fe (0.21 g). Application of *Trichoderma* could increased weight about 55% at 0 g/L Fe. It means that interaction effects were the less effective for flower dry weight. Fe treatments up to 0.75 g/L were observed simulative for growth and flower dry weight, but higher levels decreased this trait which could be considered as a toxic influence (Fig. 9).

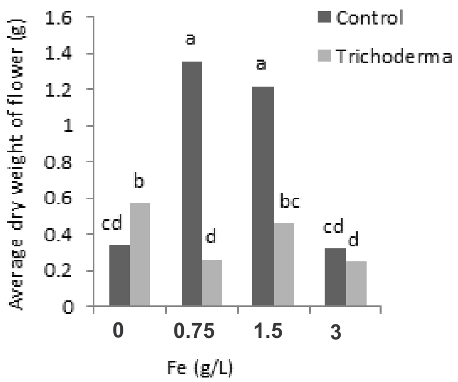

Fig. 9. Effect of interaction between *Trichoderma* and Fe on average dry weight of flower

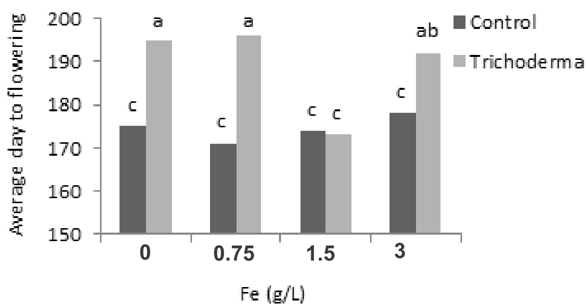

Fig. 10. Effect of interaction between *Trichoderma* and Fe on average day to flowering

Table 1. Effect of *Trichoderma harzianum* Bi and different levels of Fe on average leaf area

C.V.	Leaf area
Trichoderma harzianum Bi (w/w)	
0	35.30b
8%	58.89a
Fe (g/L)	
0	51.31a
0.75	38.97a
1.5	51.16a
3	46.93a

Means with different letter have significant difference at $p<0.01$, Tukey HSD range test.

Day to flowering

Applying *Trichoderma* and Fe had a remarkable effect on day to flowering. Minimum time to flowering (170 days) was observed in 0.75 g/L Fe, whereas maximum (195 days) was related to *Trichoderma* and 0.75 g/L Fe interaction. Rising Fe treatments showed no significant increase, but adding *Trichoderma* had a noticeable increase, except at 1.5 g/L. It generally means that interaction effects were the most effective for day to flowering in this experiment. Fe treatments up to 0.75 were observed simulative, but higher concentrations decreased this trait which could be considered as a toxic influence (Fig. 10).

DISCUSSION

In this experiment, was observed *Trichoderma* (Bi) could significantly increase more morphological traits of *Spathiphyllum*. such as leaf number, sucker number, fresh and dry weight of leaf, sucker, root, flower and number day to flowering. Fresh weight, dry weight and leaf area of *Spathiphyllum* as well as seedling weight of cabbages were increased significantly by the application of *Trichoderma* (Poldma *et al.*, 2000). The ability of *Trichoderma* to produce growth hormones (Windham *et al.*, 1986), solubilization of insoluble minor nutrients in soil (Altomare *et al.*, 1999), uptake and translocation of less-available minerals (Inbar *et al.*, 1994; Kleifeld and Chet, 1992), increased root area allowing the roots to explore larger amount 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), could be caused of the significant differences we observed in this study. Dry weight of leaf, sucker and root in the present work was significantly increased which is contrary to the findings of Yeidia *et al.* (2001). The effect of *Trichoderma* on leaf number was significant either. In most cases, increasing Fe concentration reduced traits, which could be considered as a toxic influence. Generally 0 g/lit Fe were most effective in improving the growth characteristics. Although Fe is an essential element for plants, Fe excess is believed to generate oxidative stress (Halliwell and Gutteridge, 1984). Toxic reduced O_2, species are inevitable by-products of biological oxidations. The toxicity of the relatively non-reactive superoxide radicals and H_2O_2, arises by the Fe-dependent conversion into the extremely reactive hydroxyl radicals (Haber-Weiss reaction) that cause severe damage on membranes, proteins, and DNA (Halliwell and Gutteridge, 1984).

CONCLUSION

In conclusion, using *Trichoderma* (Bi) could increase most studied characteristics of *spathiphyllum* in this experiment and improve rate of growth and development in plant. Also, different levels of Fe increased most studied traits in this experiment. Based on the results of this study, *Trichoderma* (Bi) and 0g/L Fe had better results in improved traits of *Spathiphyllum*.

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